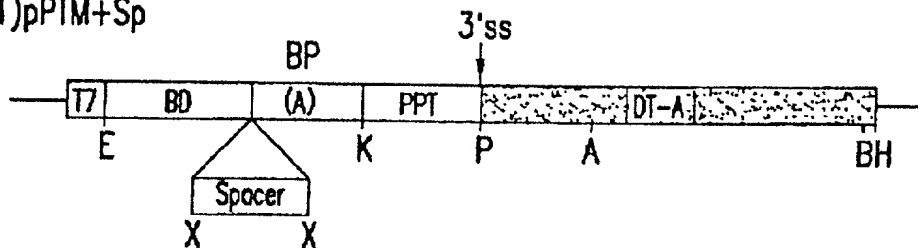


FIG.1A

(1)pPTM+Sp



(2)pPTM+Sp

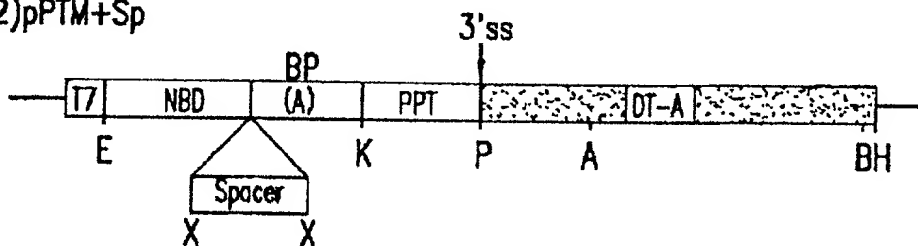


FIG.1B

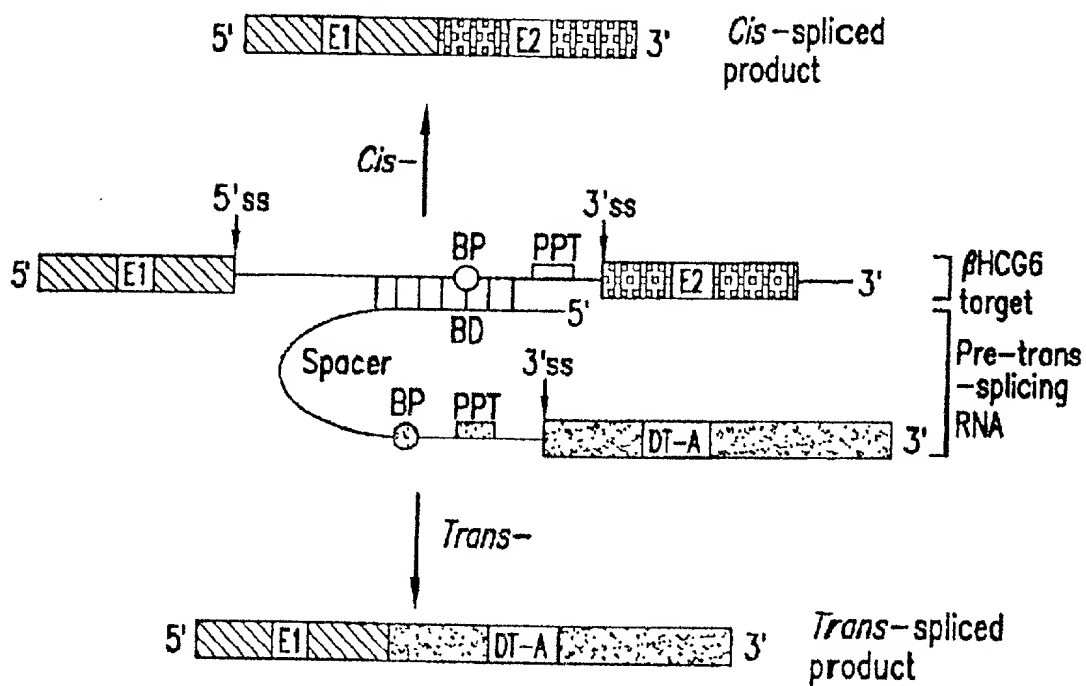


FIG.1C

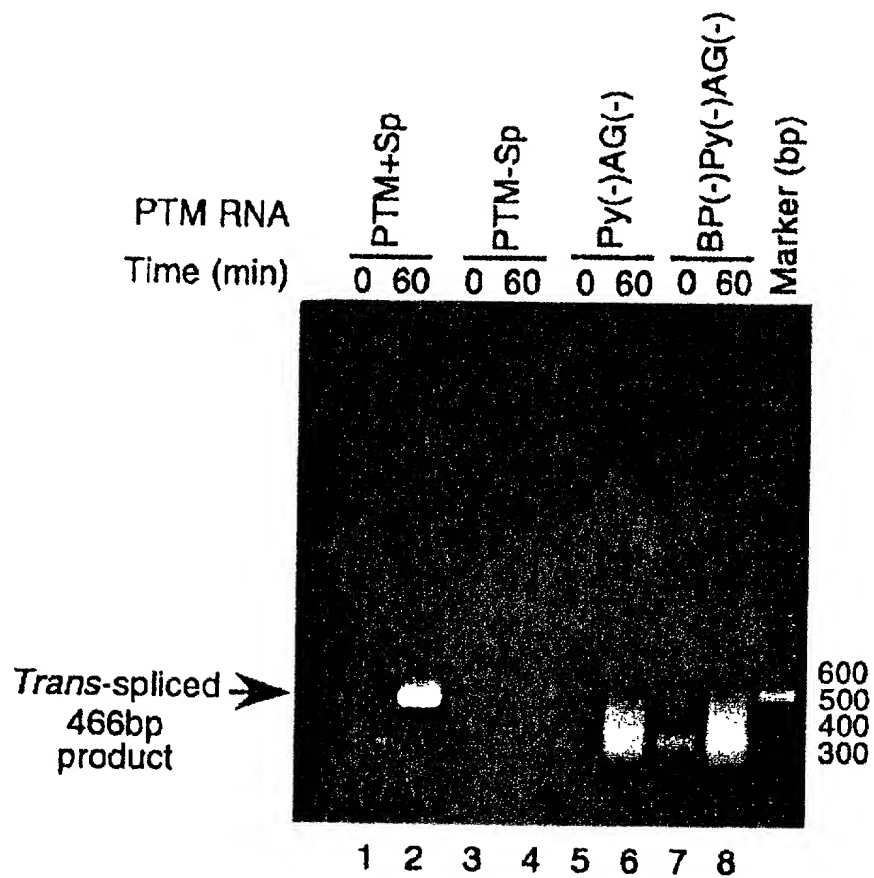


FIG.2A

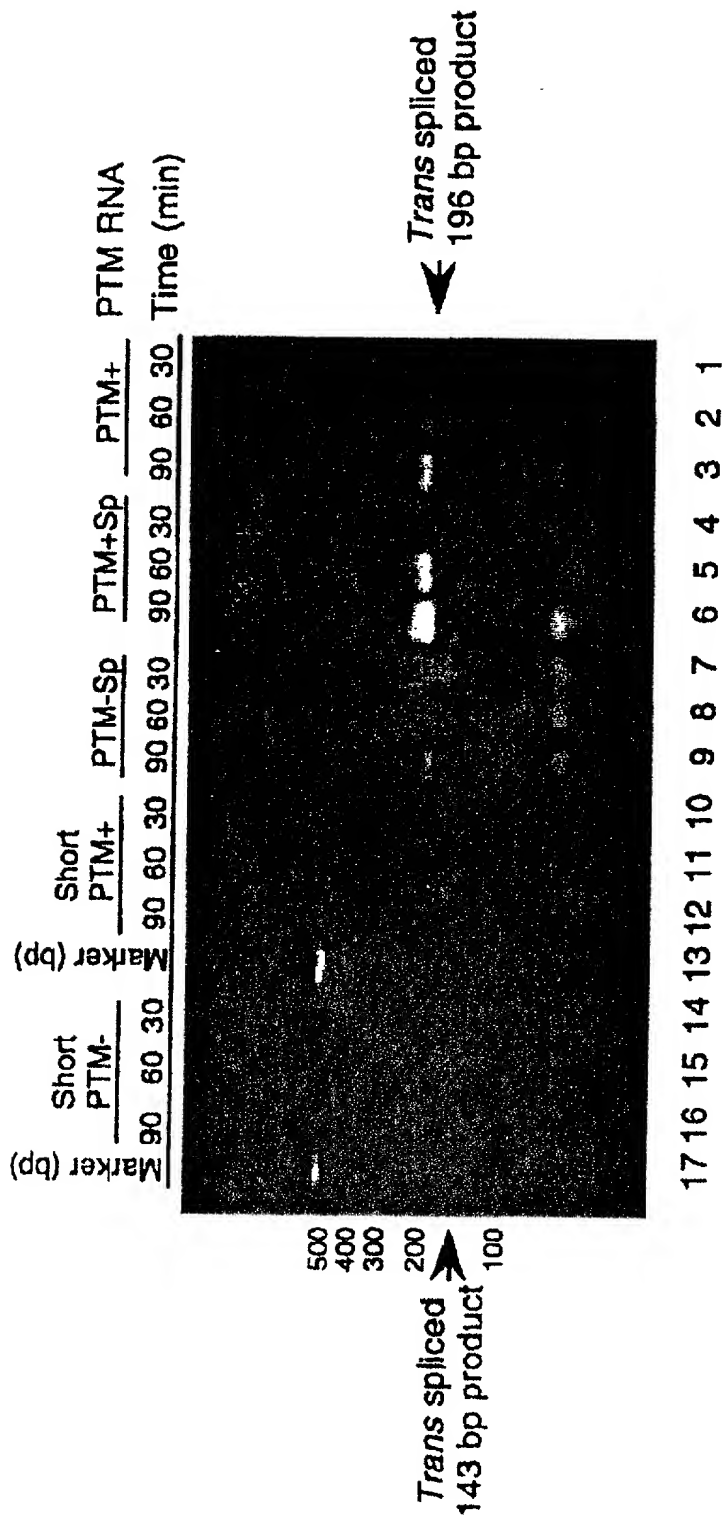


FIG.2B

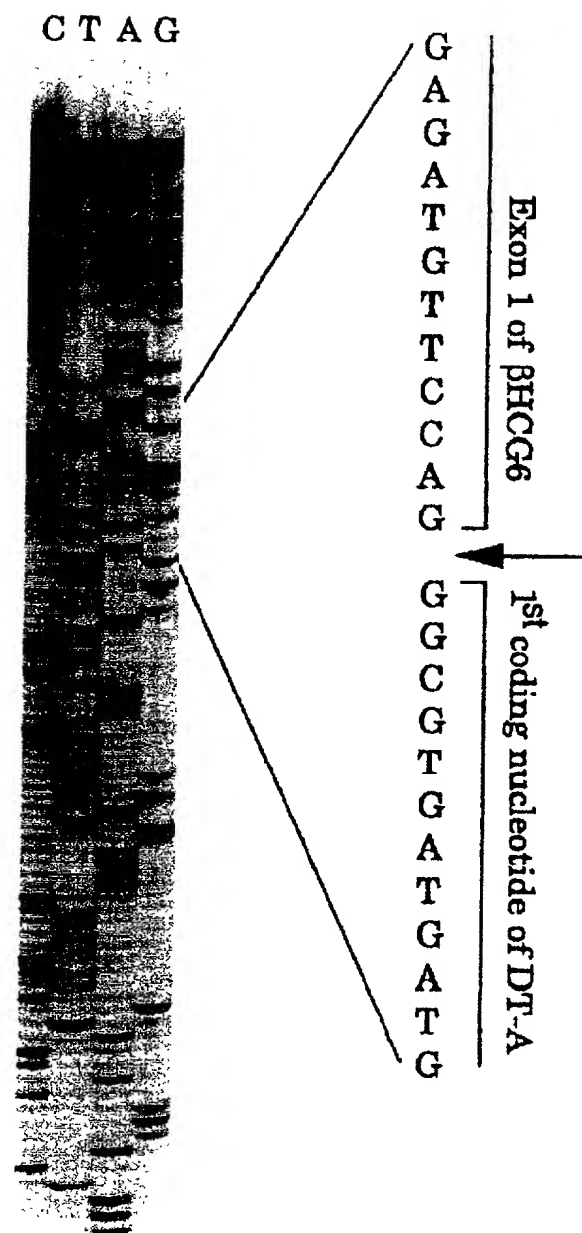
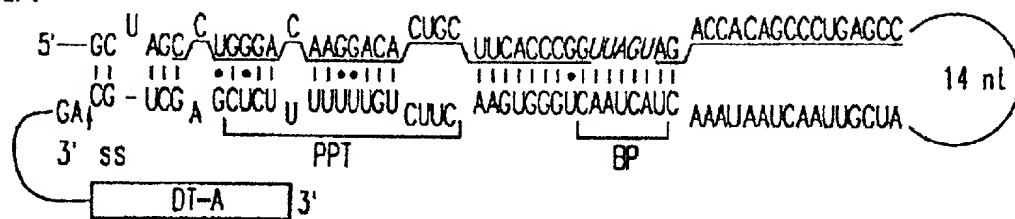


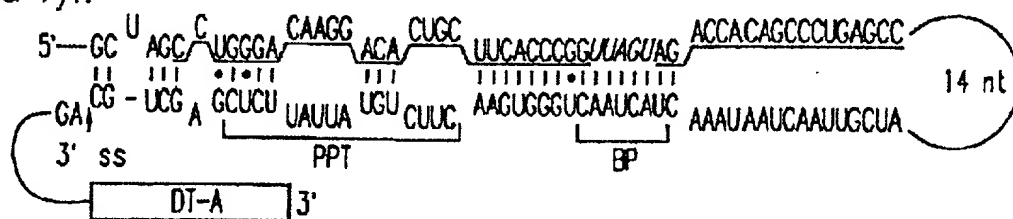
FIG.3

Year	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024	2025	2026	2027	2028	2029	2030	2031	2032	2033	2034	2035	2036	2037	2038	2039	2040	2041	2042	2043	2044	2045	2046	2047	2048	2049	2050	2051	2052	2053	2054	2055	2056	2057	2058	2059	2060	2061	2062	2063	2064	2065	2066	2067	2068	2069	2070	2071	2072	2073	2074	2075	2076	2077	2078	2079	2080	2081	2082	2083	2084	2085	2086	2087	2088	2089	2090	2091	2092	2093	2094	2095	2096	2097	2098	2099	2100
1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024	2025	2026	2027	2028	2029	2030	2031	2032	2033	2034	2035	2036	2037	2038	2039	2040	2041	2042	2043	2044	2045	2046	2047	2048	2049	2050	2051	2052	2053	2054	2055	2056	2057	2058	2059	2060	2061	2062	2063	2064	2065	2066	2067	2068	2069	2070	2071	2072	2073	2074	2075	2076	2077	2078	2079	2080	2081	2082	2083	2084	2085	2086	2087	2088	2089	2090	2091	2092	2093	2094	2095	2096	2097	2098	2099	2100	

1. PTM+SF:



2. PTM+SF-Py1:



3. PTM+SF-Py2:

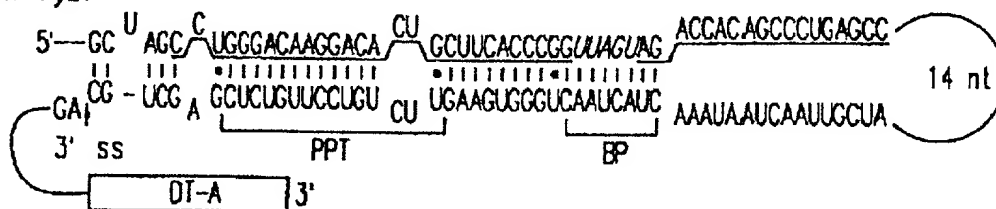


FIG.4A

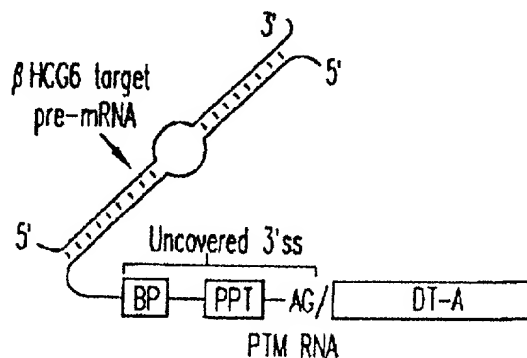


FIG.4B

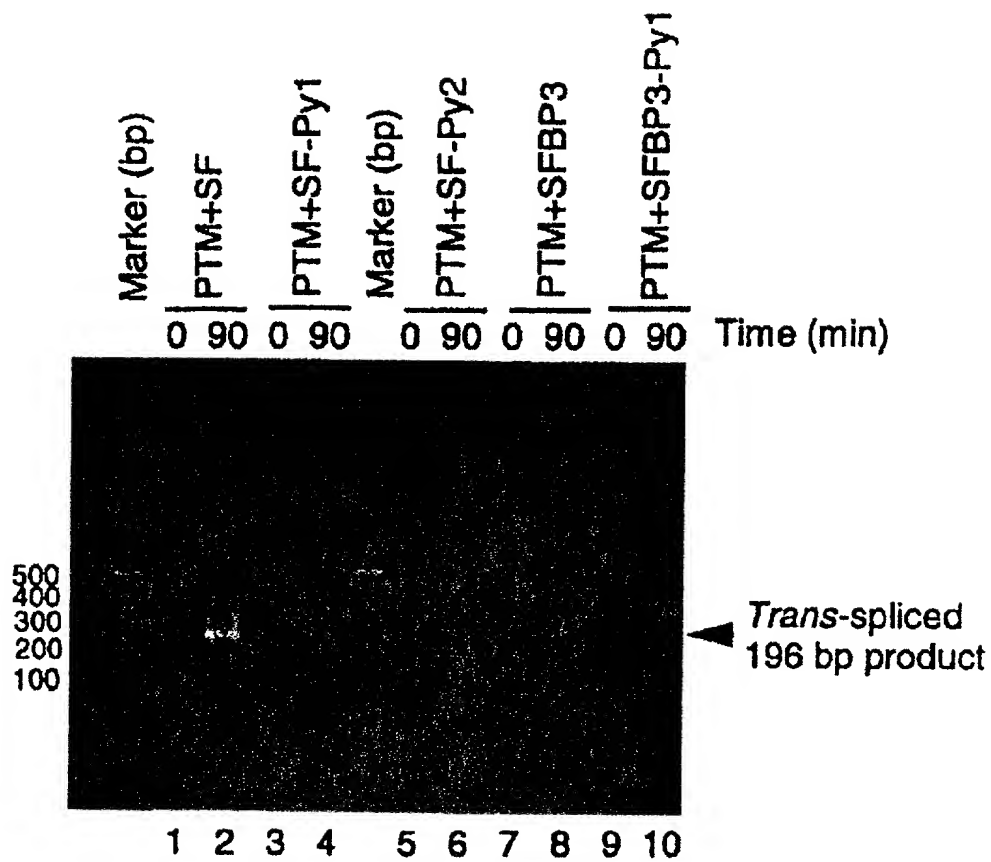


FIG.4C



FIG. 5

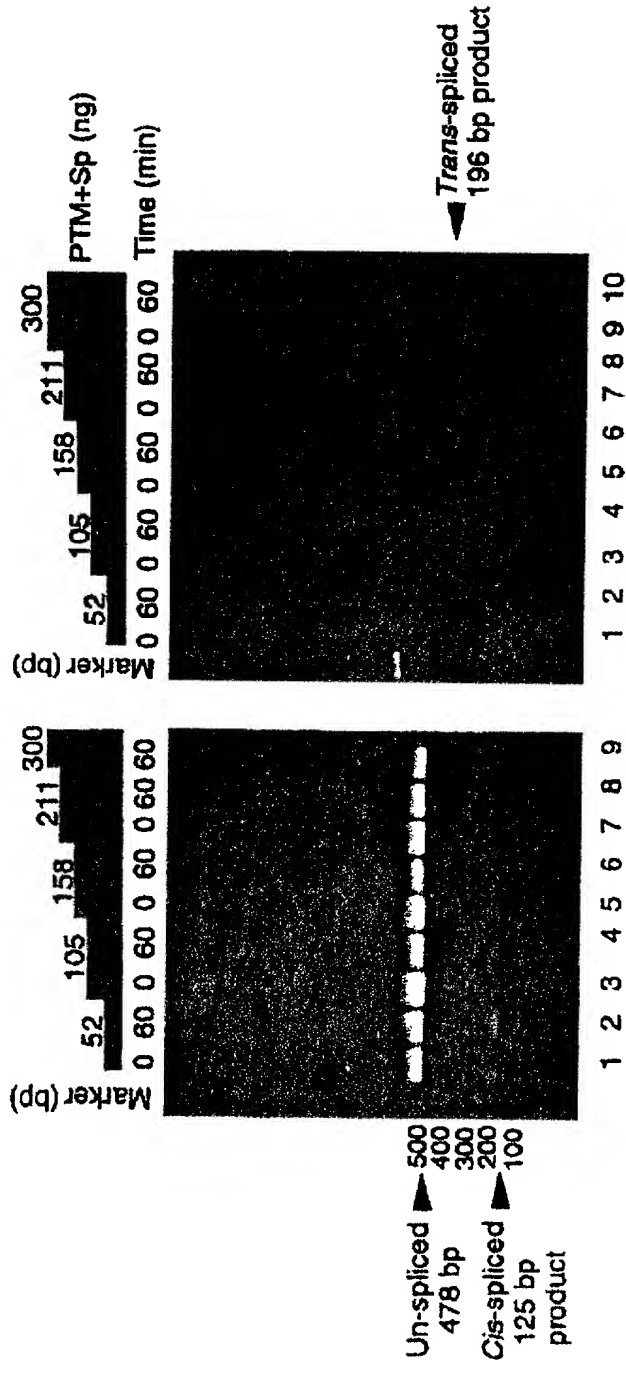


FIG. 6A

FIG. 6B

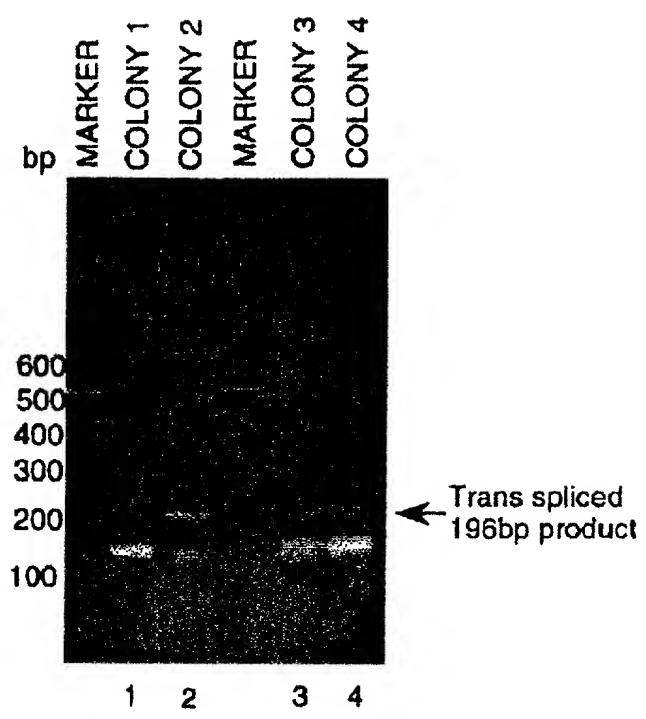


FIG.7A

EXON 1 OF β HCG6 ↓
5'-CAGGGACGCACCACCAAGGATGGAGA**GTCCTCCAG-GGGCTGATGATTGTT**
↓ 1ST CODING NUCLEOTIDE OF DT-A
GATTCCTCTTAATCCTTTGTGATGGAAACTTTCCTTCGTACCAACGGGACTA
AACCTGGTTATGTAGATTCATTCAAAA-3'

FIG. 7B

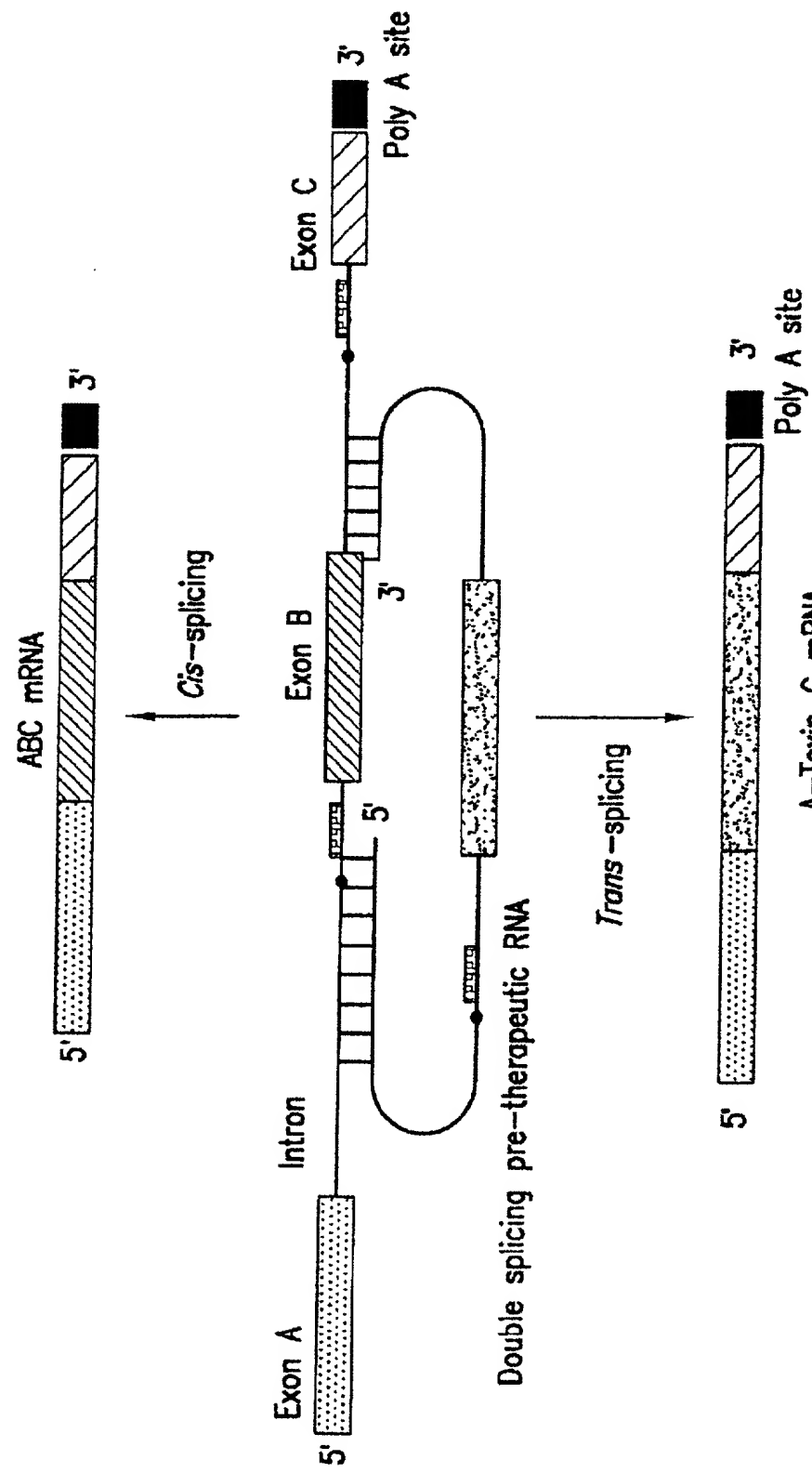
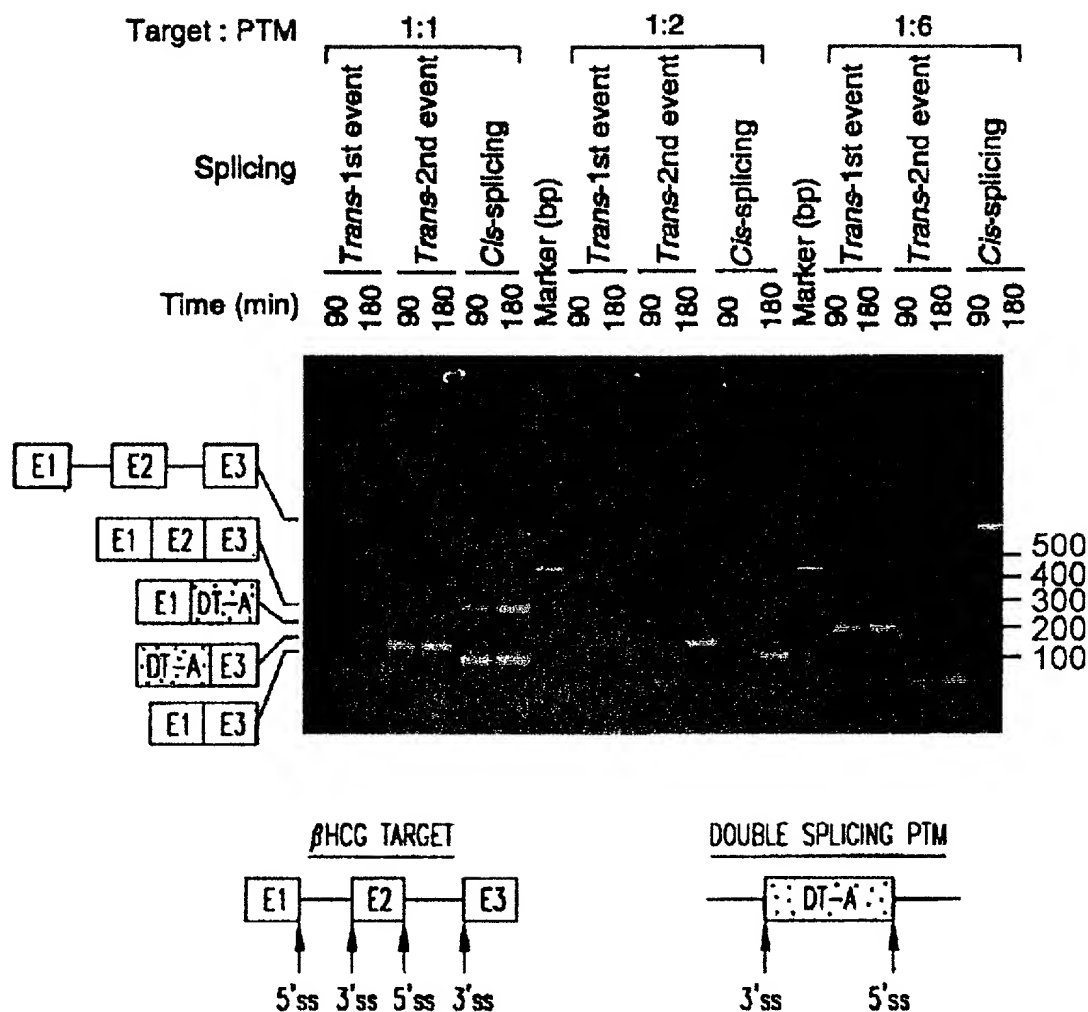


FIG.8A



Cis-spliced products

E1 E2 E3 = NORMAL *cis*-SPLICING (277bp)

E1 E3 = Exon SKIPPING (110bp)

Trans-spliced products

E1 DT-A = 1st EVENT, 196bp. *Trans*-SPLICING BETWEEN 5' ss OF TARGET & 3' ss OF PTM.

DT-A E3 = 2nd EVENT, 161bp. *Trans*-SPLICING BETWEEN 3' ss OF TARGET & 5' ss OF PTM.

FIG.8B

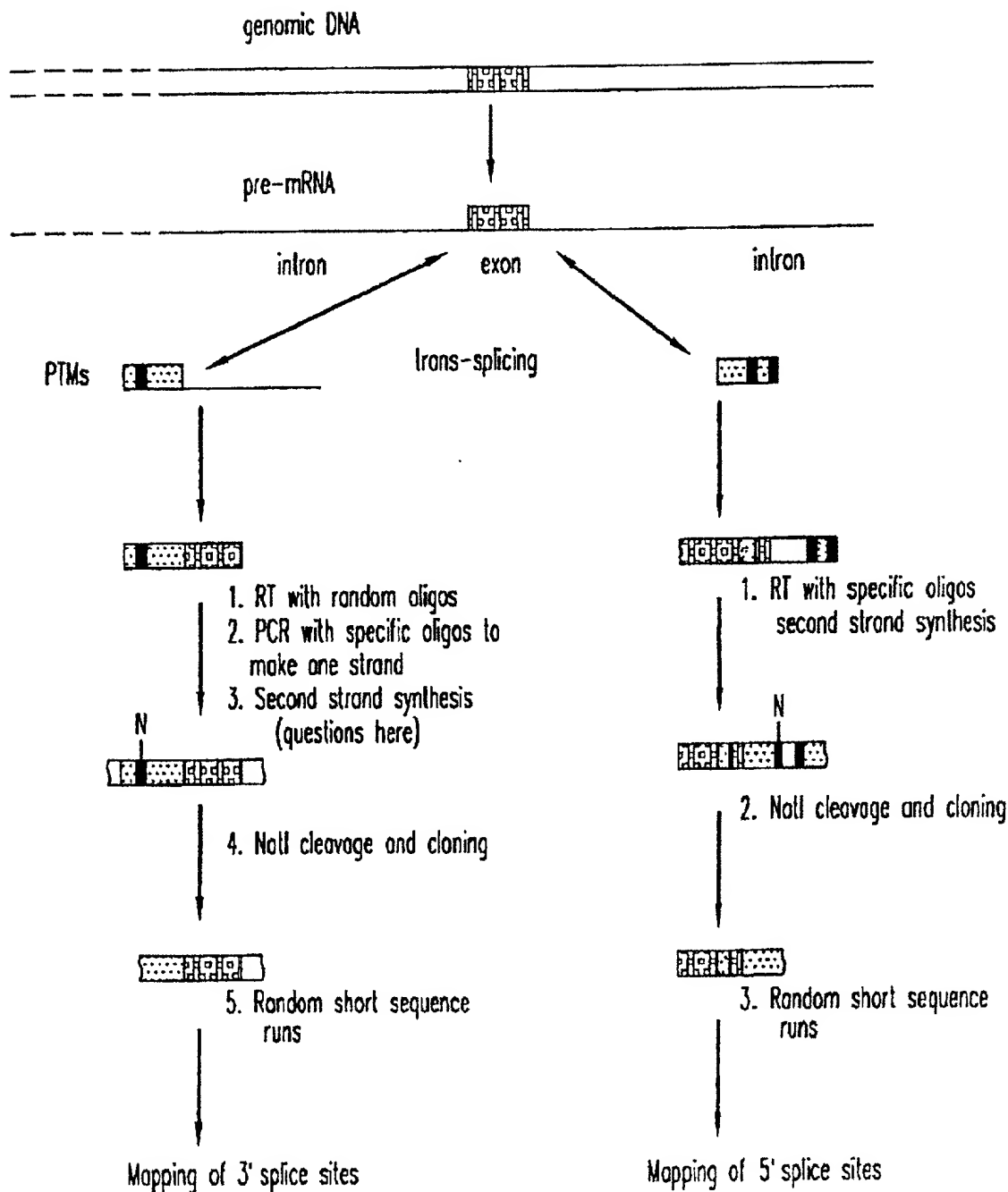
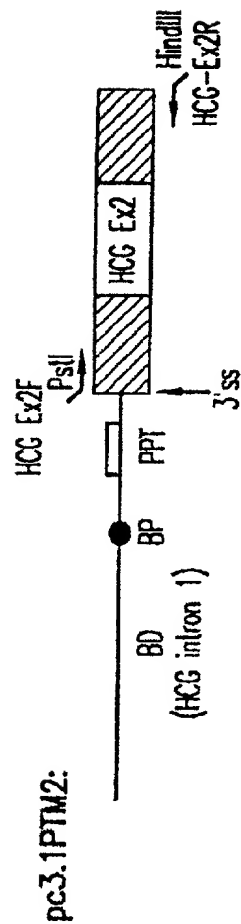
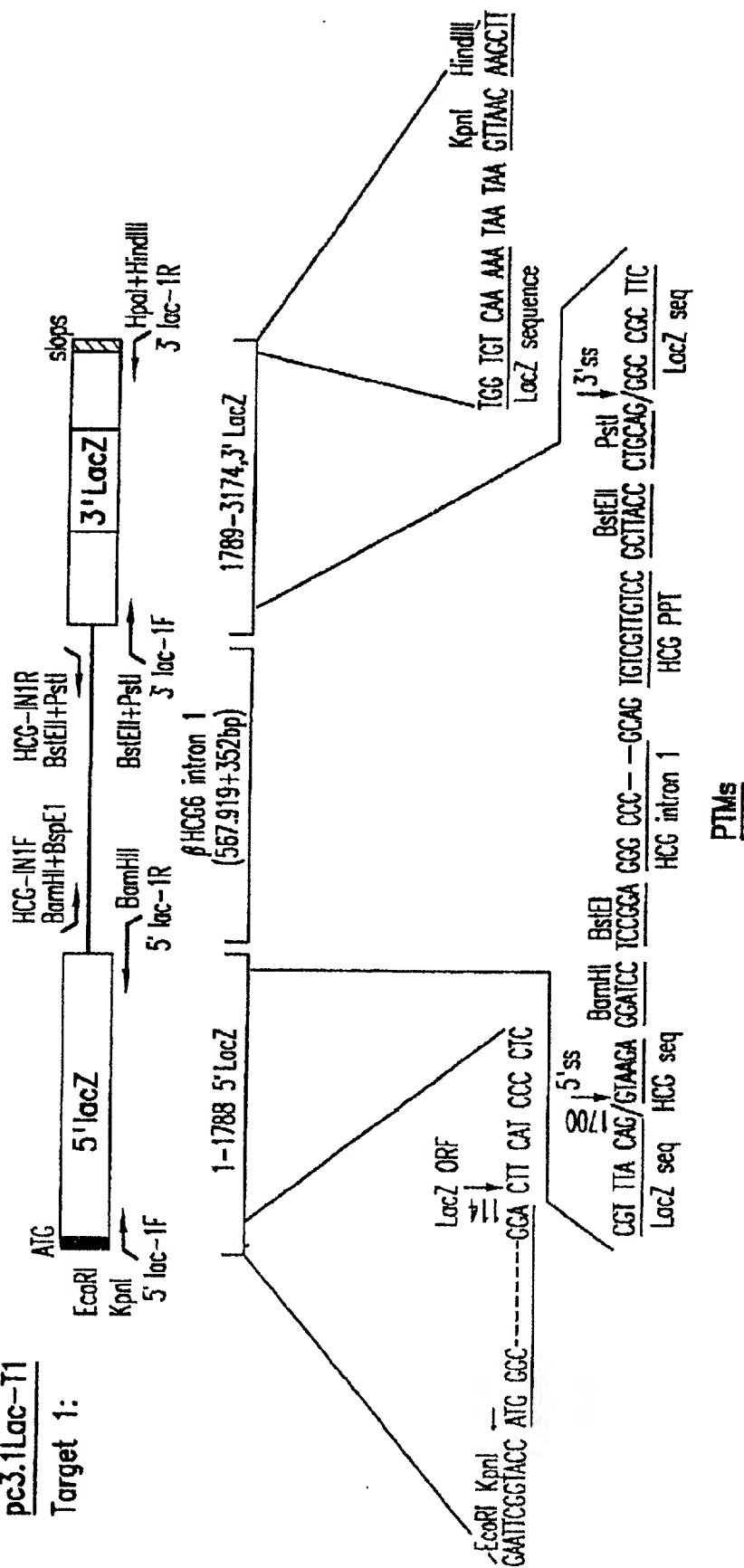


FIG.9

pc3.1Lac-T1

Target 1:



pc3.1PTM2:

FIG.10A

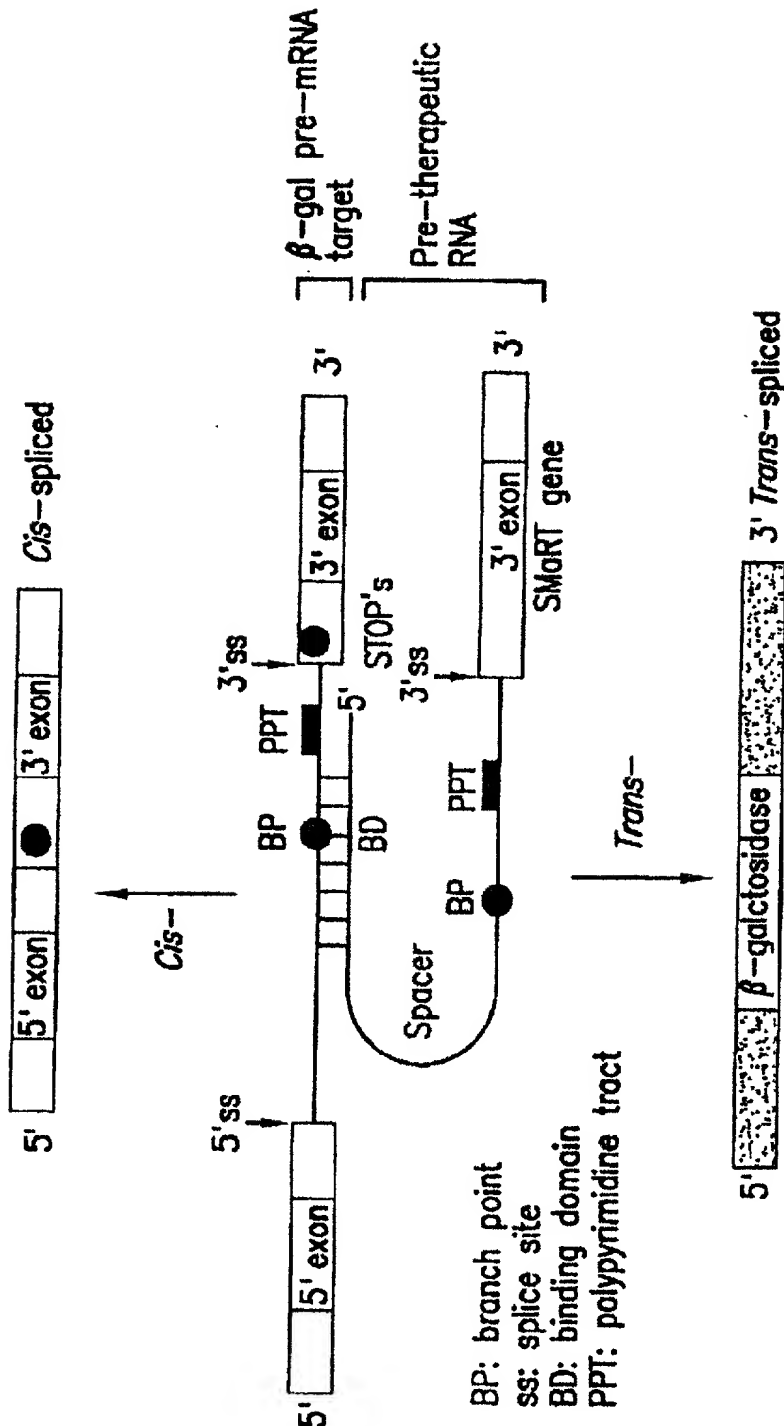


FIG.10B

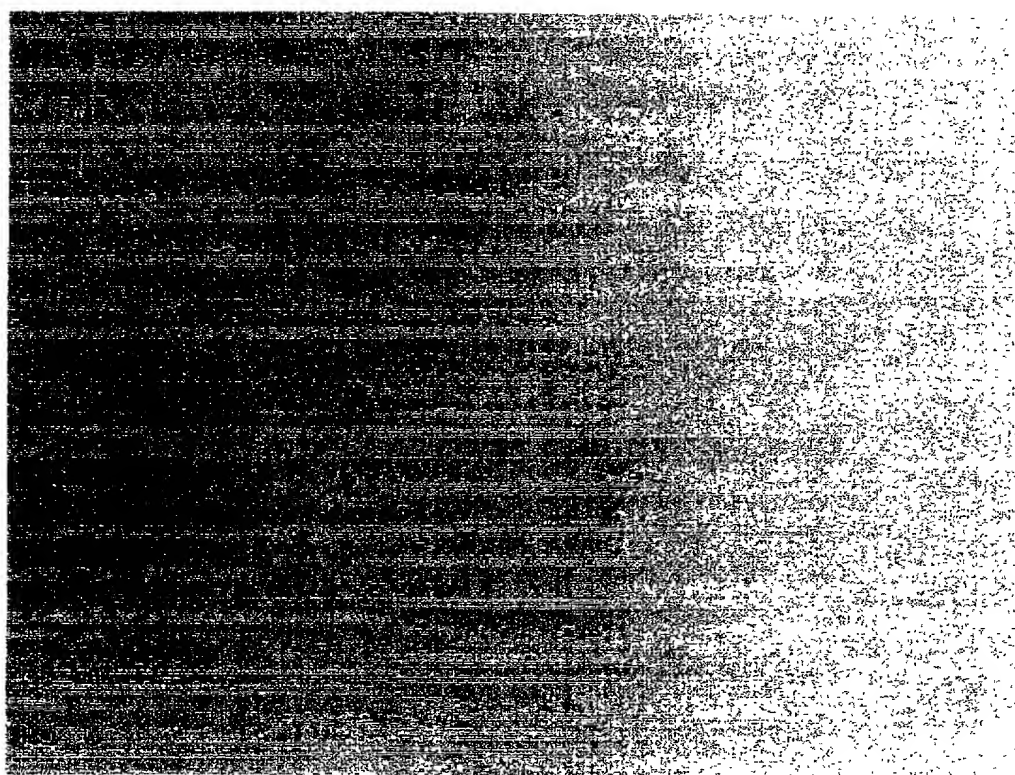


FIG.11B

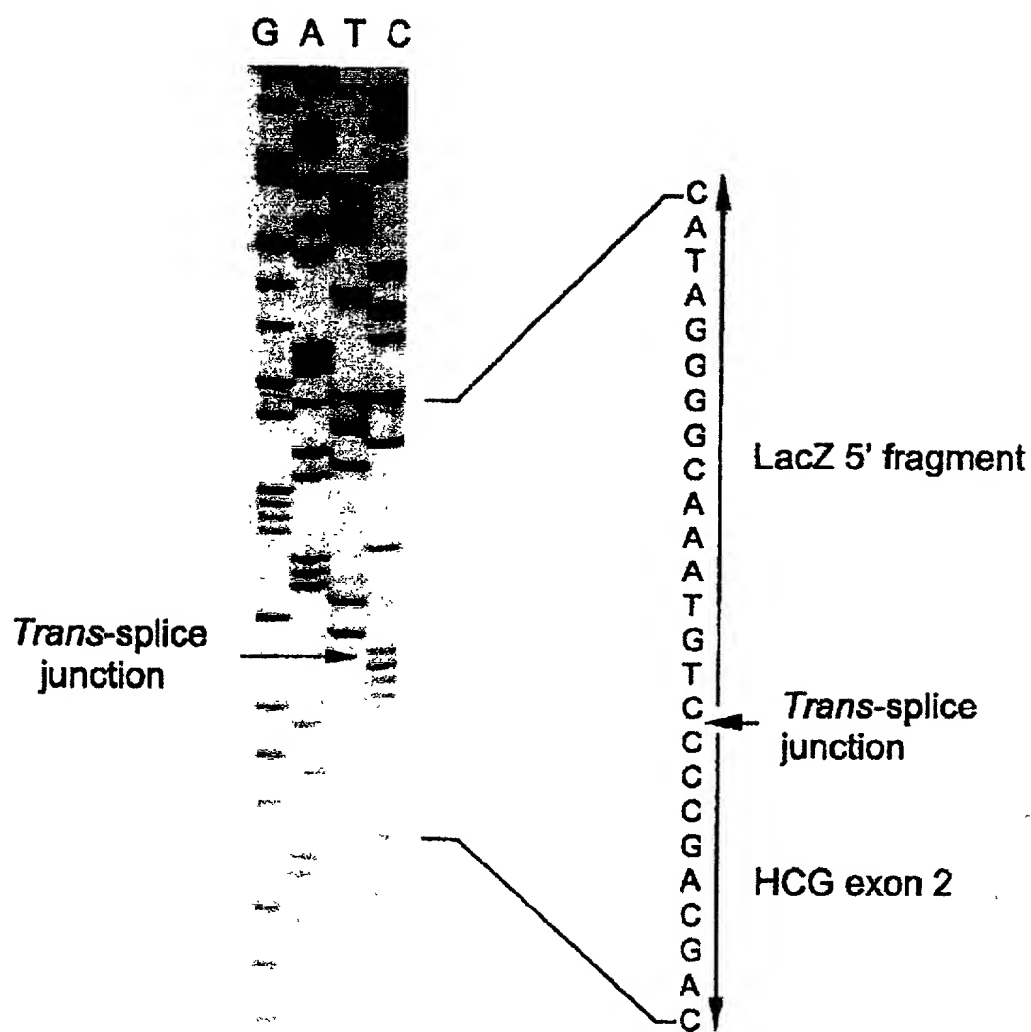


FIG.12A

1. NUCLEOTIDE SEQUENCES OF THE *cis*-SPICED PRODUCT (285 bp):

BioLac-TR1

GGCTTTGGCTACCTGGAGAGACGGCCGGCTGATCCTTTGGGAATACGCCACCGGATGGGTAAACAGTCTTG

Splice junction

GGGGTTGGCTAAATACTGGCAGGGGTTTGGTCAGTATCCCGTTTACAG/GGGGGCTTGGTCTAATAATG

GGACTGGGTGGATCAGTCGCTGATTAAATATGATGAACAACCGGACCGTGGTGGCTTACGGCGGTGATTT

TGGCGATACGCCGAACGATCGCCAGTTCTGTATGAACGGTCTGGTCTTTGCCGACCGCACCGGCATCCAG

Lac-TR2

2. NUCLEOTIDE SEQUENCES OF THE *trans*-SPICED PRODUCT (195 bp)

BioLac-TR1

GGCTTTGGCTACCTGGAGAGACGGCCGGCTGATCCTTTGGGAATACGCCACCGGATGGGTAAACAGTCTTG

Splice junction

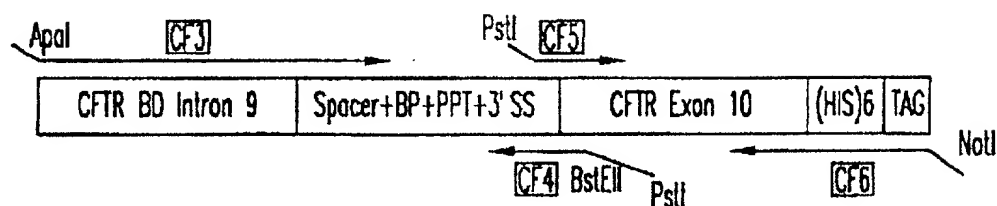
CGGTTTGGCTAAATACTGGCAGGGGTTTGGTCAGTATCCCGTTTACAG/GGGCTGCTGCTGTTGCTGCTGCT

HCGR2

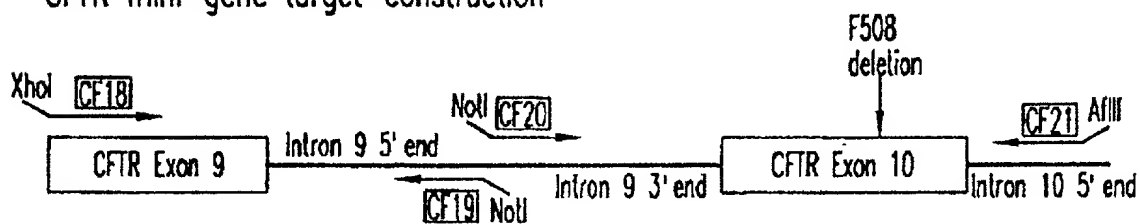
GAGCATGGCGGGACATGGGCATCCAAGGAGCCACTTCGGCCACCGGTGCGG

FIG.12B

CFTR Pre-therapeutic molecule (PTM or "bullet")



CFTR mini-gene target-construction



Trans-splicing Repair

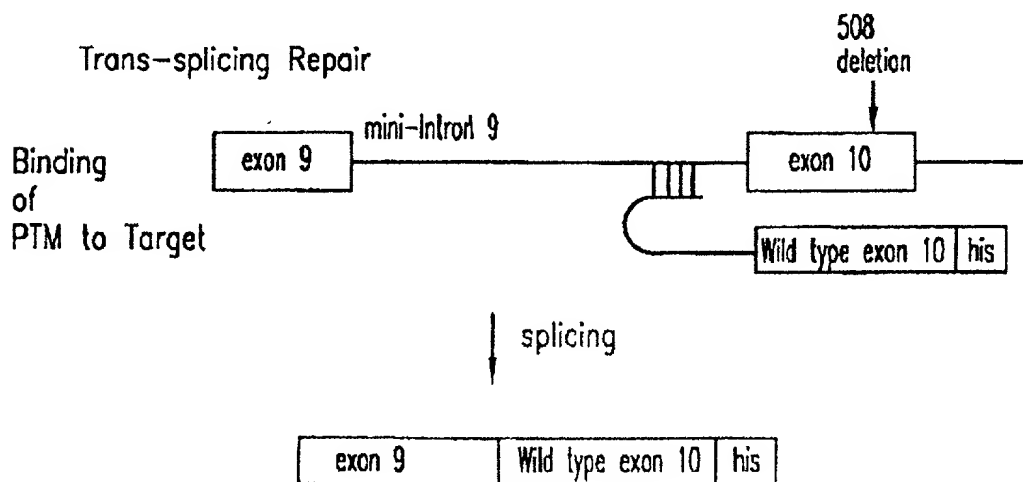


FIG.13

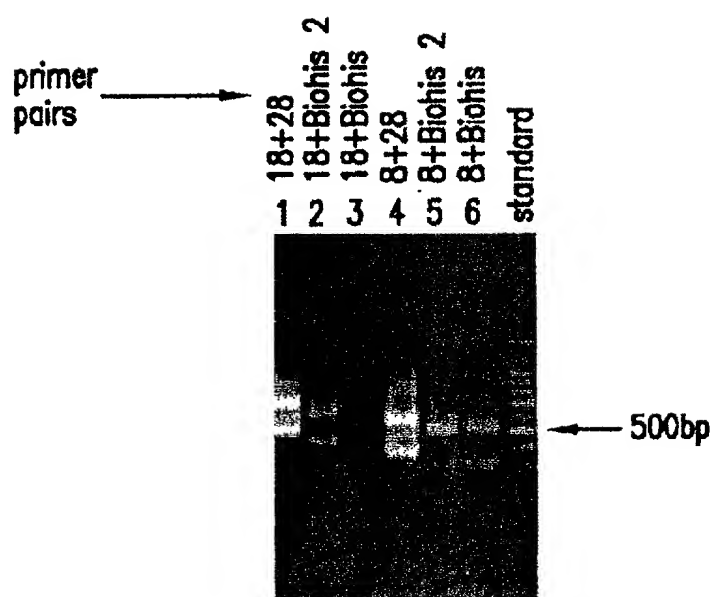


FIG.14

DNA sequence 500 b.p. GCTAGCGTTTAA ... TGCCACTCCAC linear

Positions of Restriction Endonucleases sites (unique sites underlined)

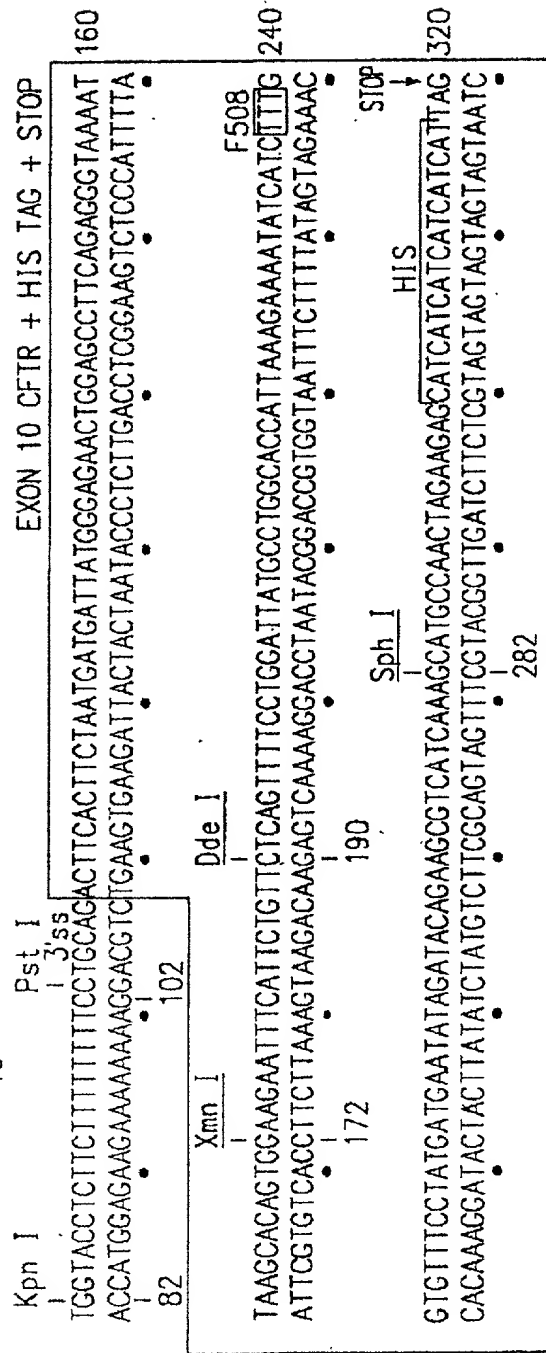
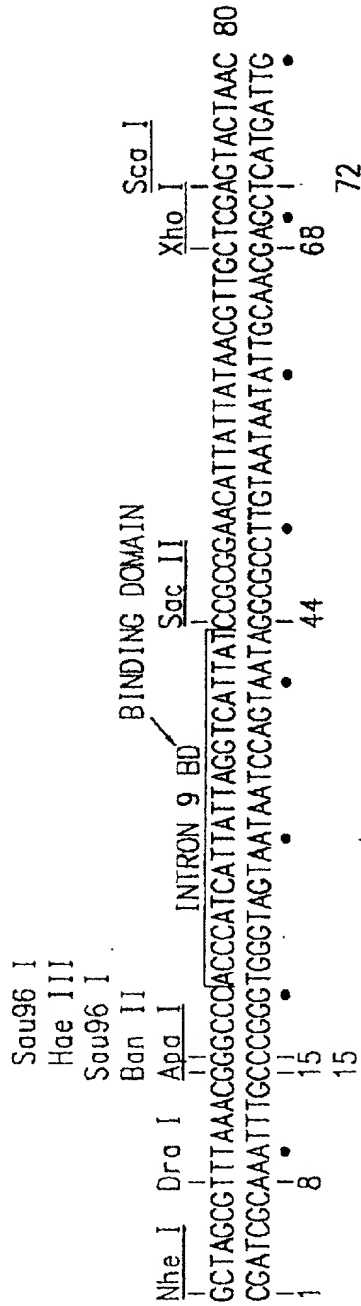
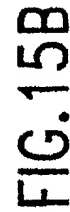


FIG.15A



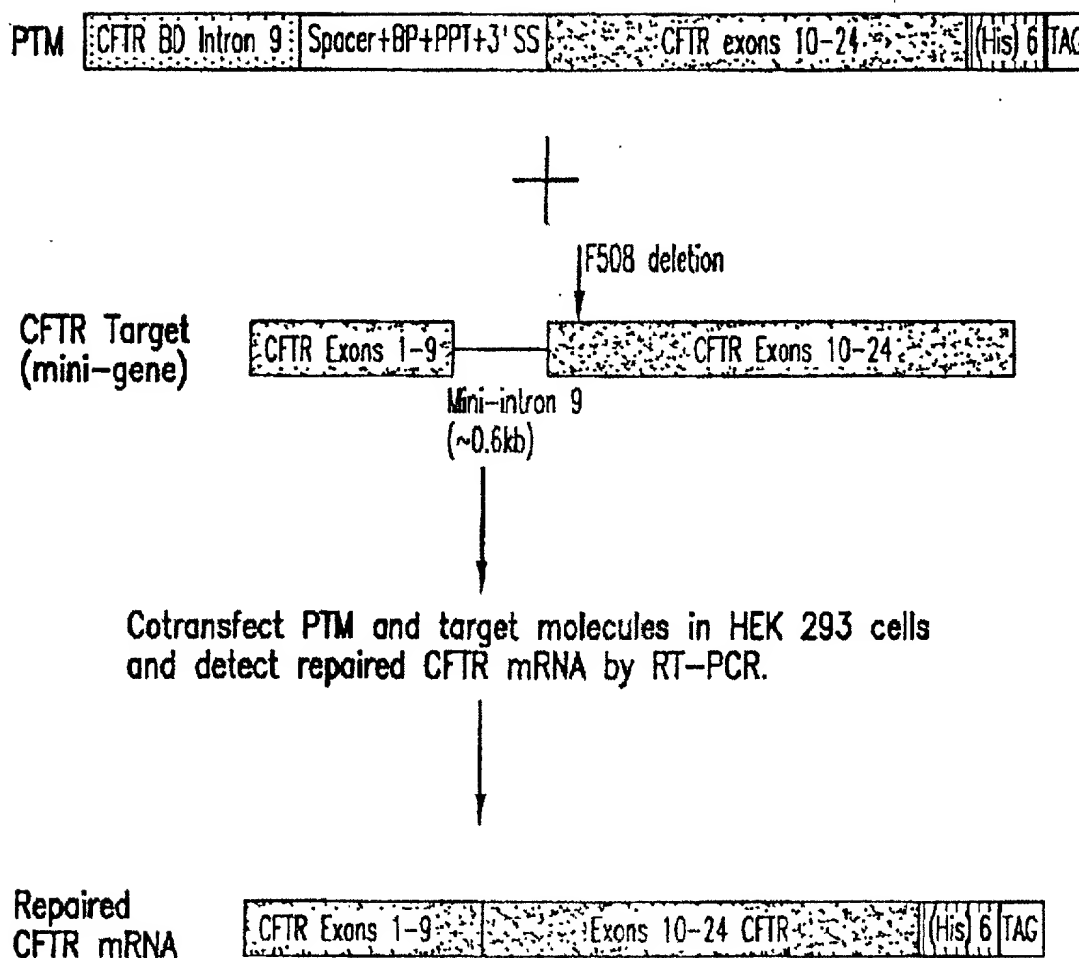


FIG.16

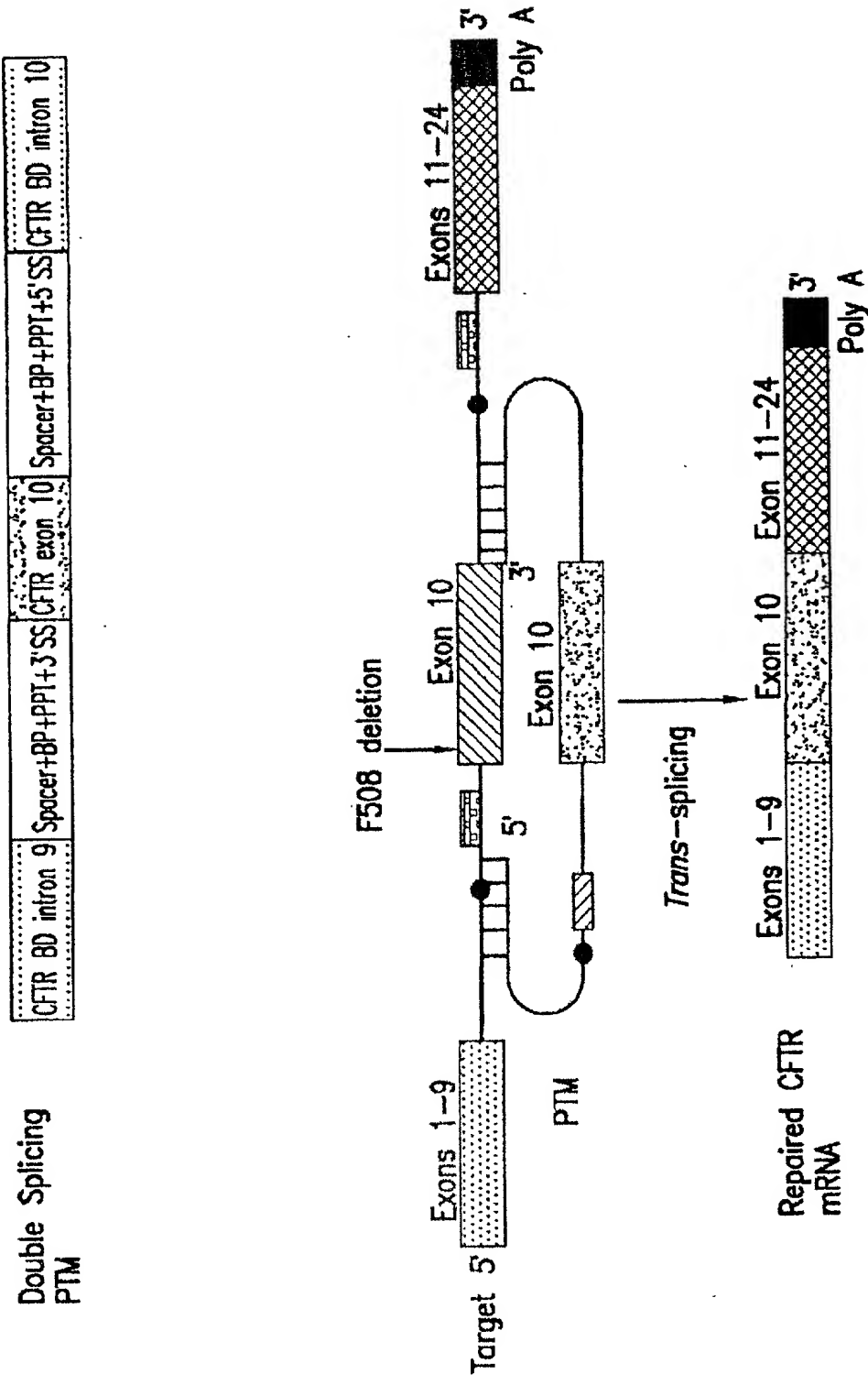


FIG.17

DOUBLE TRANS-SPLICING SPECIFIC TARGET

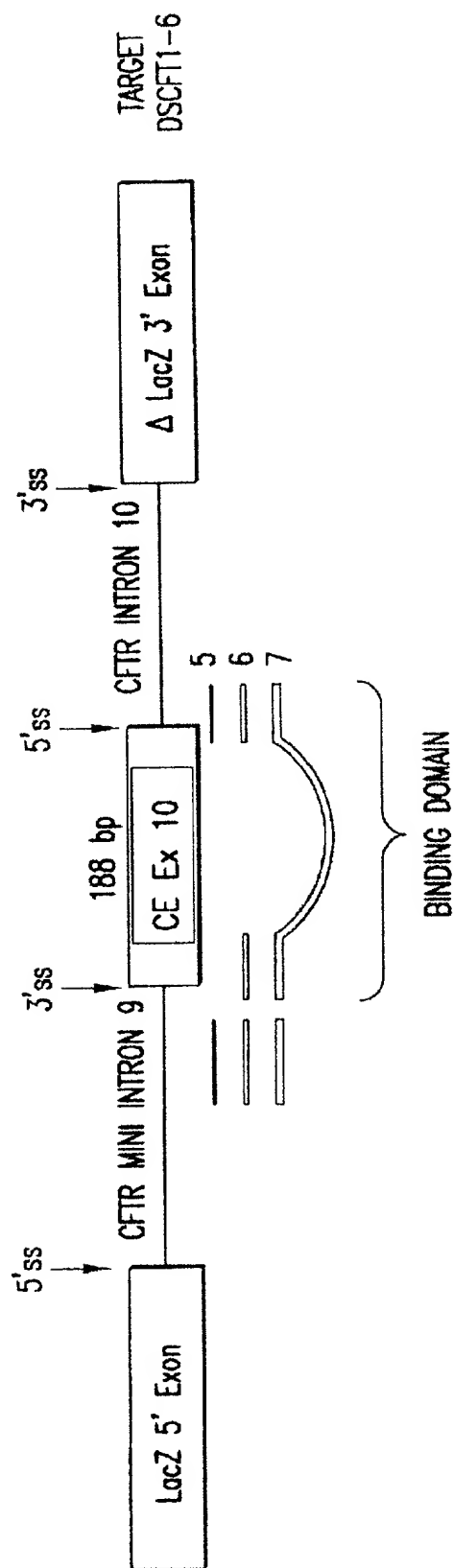


FIG.18

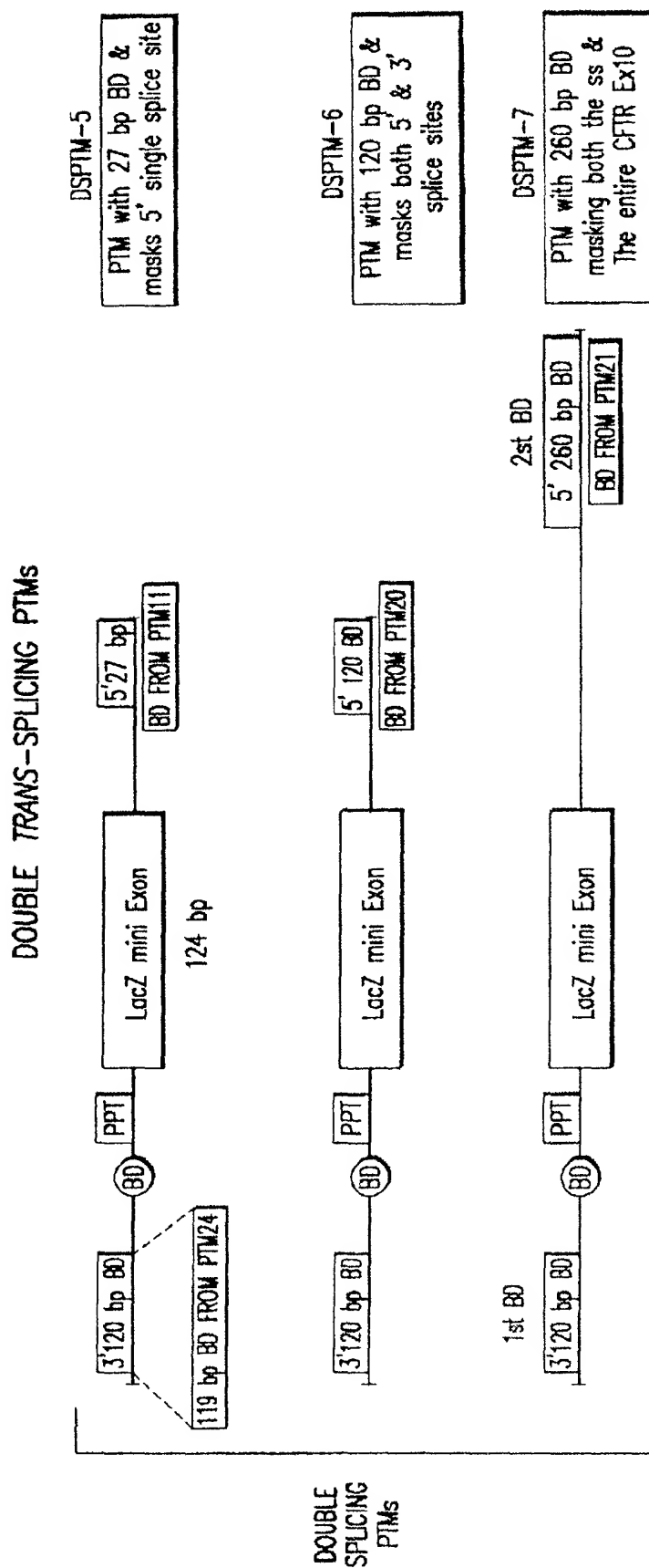


FIG.19

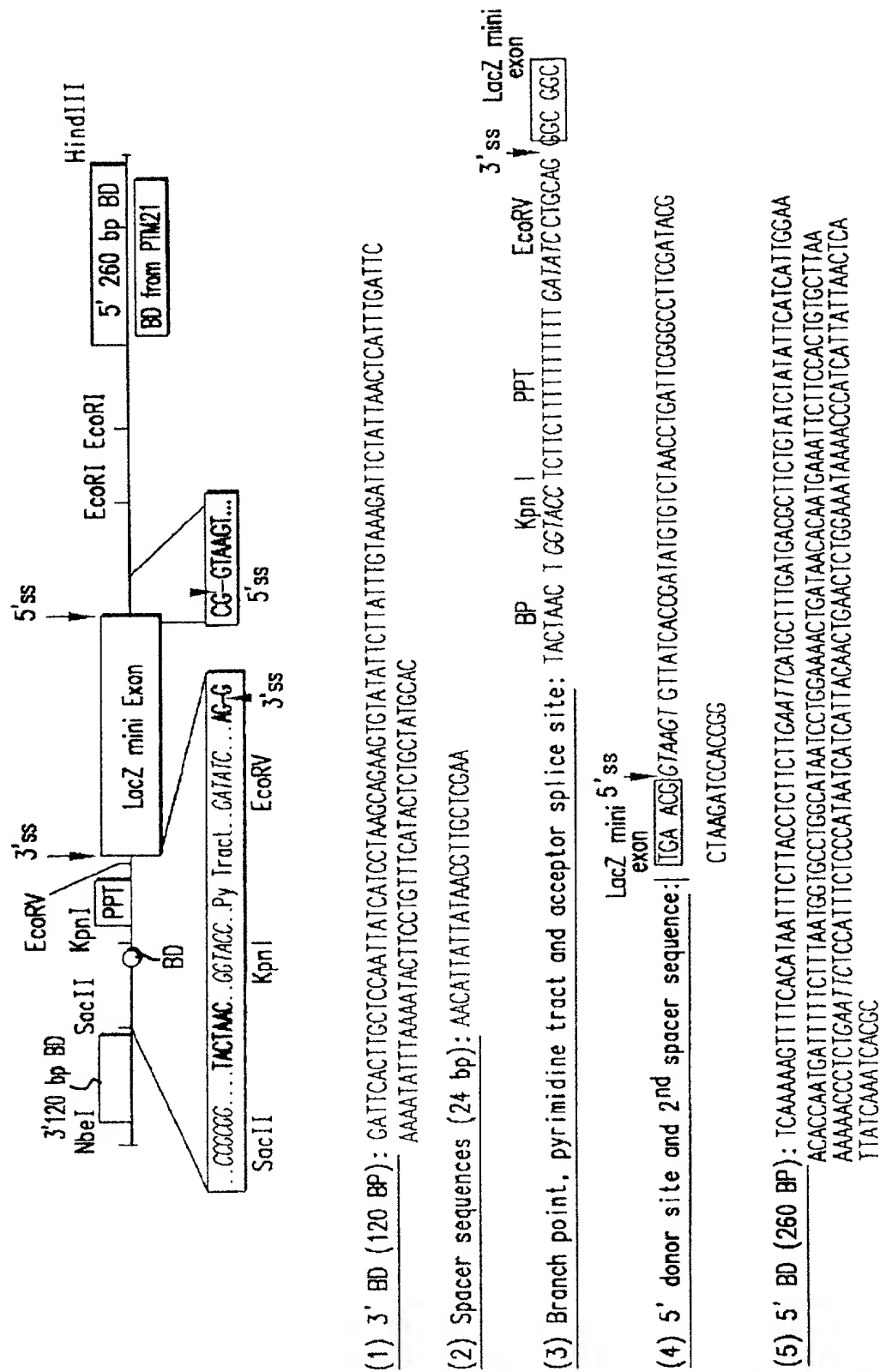
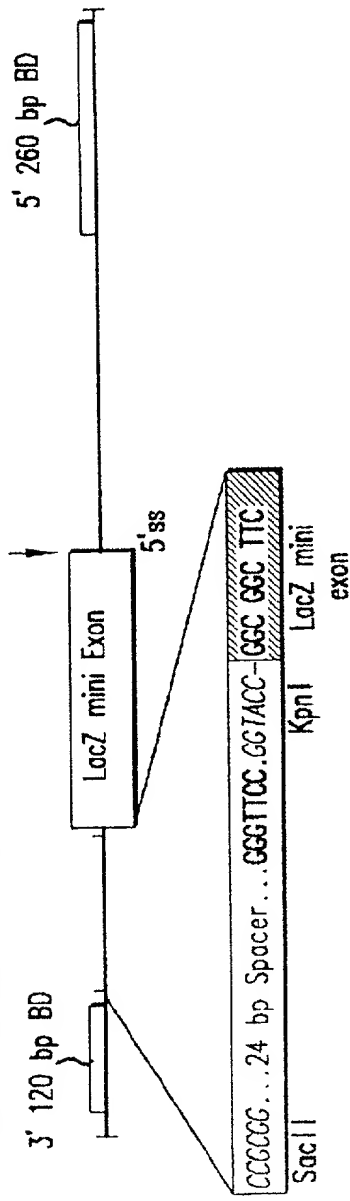
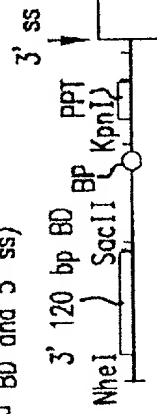


FIG.21

DSPTM8: (Δ 3' ss: 3' splice elements i.e. BP, PPT & AG dinucleotide has been deleted and replaced with random sequences, but still has the functional 5' splice site)



PTM29 (lacks 2nd BD and 5' ss)



PTM30 (lacks 1st BD and 3' ss)



FIG.22

Mutants

Case	Age	Sex	Site	Pathologic Findings	Survival
1	65	M	Rectum	Adenocarcinoma	10 months
2	68	M	Rectum	Adenocarcinoma	12 months
3	70	M	Rectum	Adenocarcinoma	18 months
4	72	M	Rectum	Adenocarcinoma	24 months
5	75	M	Rectum	Adenocarcinoma	36 months
6	78	M	Rectum	Adenocarcinoma	48 months
7	80	M	Rectum	Adenocarcinoma	60 months
8	82	M	Rectum	Adenocarcinoma	72 months
9	85	M	Rectum	Adenocarcinoma	84 months
10	88	M	Rectum	Adenocarcinoma	96 months
11	90	M	Rectum	Adenocarcinoma	108 months
12	92	M	Rectum	Adenocarcinoma	120 months
13	95	M	Rectum	Adenocarcinoma	132 months
14	98	M	Rectum	Adenocarcinoma	144 months
15	100	M	Rectum	Adenocarcinoma	156 months
16	102	M	Rectum	Adenocarcinoma	168 months
17	105	M	Rectum	Adenocarcinoma	180 months
18	108	M	Rectum	Adenocarcinoma	192 months
19	110	M	Rectum	Adenocarcinoma	204 months
20	112	M	Rectum	Adenocarcinoma	216 months
21	115	M	Rectum	Adenocarcinoma	228 months
22	118	M	Rectum	Adenocarcinoma	240 months
23	120	M	Rectum	Adenocarcinoma	252 months
24	122	M	Rectum	Adenocarcinoma	264 months
25	125	M	Rectum	Adenocarcinoma	276 months
26	128	M	Rectum	Adenocarcinoma	288 months
27	130	M	Rectum	Adenocarcinoma	300 months
28	132	M	Rectum	Adenocarcinoma	312 months
29	135	M	Rectum	Adenocarcinoma	324 months
30	138	M	Rectum	Adenocarcinoma	336 months
31	140	M	Rectum	Adenocarcinoma	348 months
32	142	M	Rectum	Adenocarcinoma	360 months
33	145	M	Rectum	Adenocarcinoma	372 months
34	148	M	Rectum	Adenocarcinoma	384 months
35	150	M	Rectum	Adenocarcinoma	396 months
36	152	M	Rectum	Adenocarcinoma	408 months
37	155	M	Rectum	Adenocarcinoma	420 months
38	158	M	Rectum	Adenocarcinoma	432 months
39	160	M	Rectum	Adenocarcinoma	444 months
40	162	M	Rectum	Adenocarcinoma	456 months
41	165	M	Rectum	Adenocarcinoma	468 months
42	168	M	Rectum	Adenocarcinoma	480 months
43	170	M	Rectum	Adenocarcinoma	492 months
44	172	M	Rectum	Adenocarcinoma	504 months
45	175	M	Rectum	Adenocarcinoma	516 months
46	178	M	Rectum	Adenocarcinoma	528 months
47	180	M	Rectum	Adenocarcinoma	540 months
48	182	M	Rectum	Adenocarcinoma	552 months
49	185	M	Rectum	Adenocarcinoma	564 months
50	188	M	Rectum	Adenocarcinoma	576 months
51	190	M	Rectum	Adenocarcinoma	588 months
52	192	M	Rectum	Adenocarcinoma	600 months
53	195	M	Rectum	Adenocarcinoma	612 months
54	198	M	Rectum	Adenocarcinoma	624 months
55	200	M	Rectum	Adenocarcinoma	636 months
56	202	M	Rectum	Adenocarcinoma	648 months
57	205	M	Rectum	Adenocarcinoma	660 months
58	208	M	Rectum	Adenocarcinoma	672 months
59	210	M	Rectum	Adenocarcinoma	684 months
60	212	M	Rectum	Adenocarcinoma	696 months
61	215	M	Rectum	Adenocarcinoma	708 months
62	218	M	Rectum	Adenocarcinoma	720 months
63	220	M	Rectum	Adenocarcinoma	732 months
64	222	M	Rectum	Adenocarcinoma	744 months
65	225	M	Rectum	Adenocarcinoma	756 months
66	228	M	Rectum	Adenocarcinoma	768 months
67	230	M	Rectum	Adenocarcinoma	780 months
68	232	M	Rectum	Adenocarc	

LacZ 5' Exon

LacZ Mini Exon

	10	20	30	40	50	60	70
LOC. 3 EAST							
	TTTAT	CCCGG	CTTC	GGGCT	GGAT	CAGT	CGCT
	ATA	TTAC	AGGG	CGG	CTGG	CAAT	AAAT
	AT	CA	GA	TA	AT	CA	TA
	GA	AA	AT	CA	TA	GA	AA

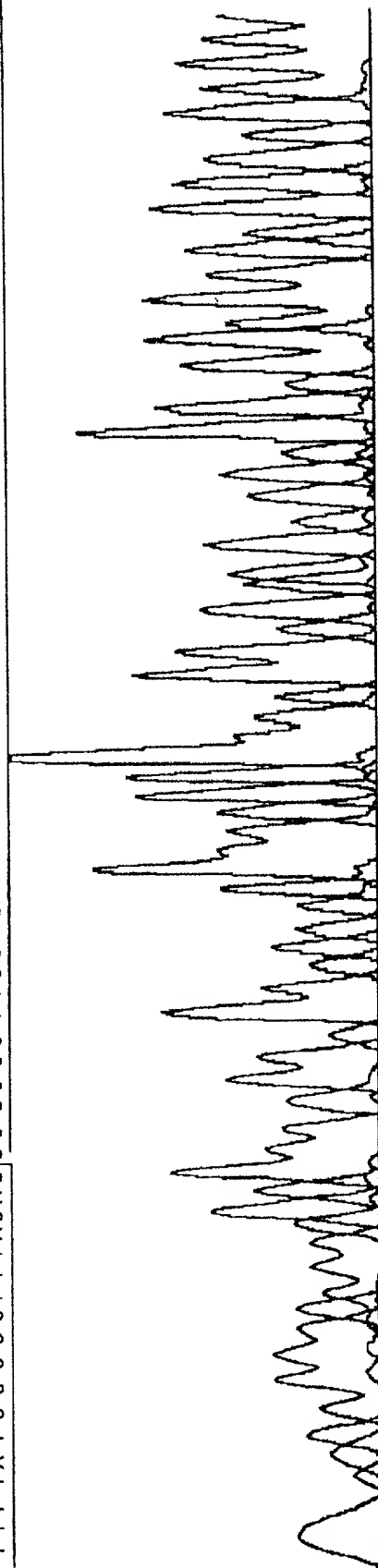


FIG. 23A

ACCURACY OF DOUBLE TRANS-SPLICING REACTION

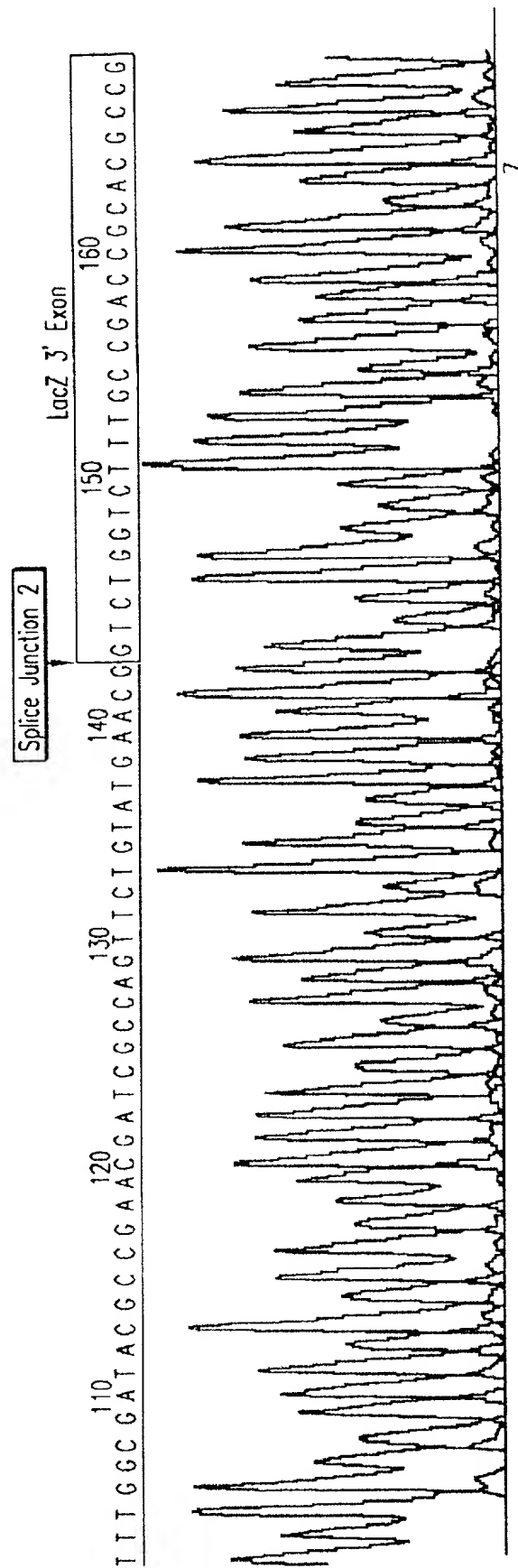


FIG.23B

Double Trans-splicing Produces Full-length Protein

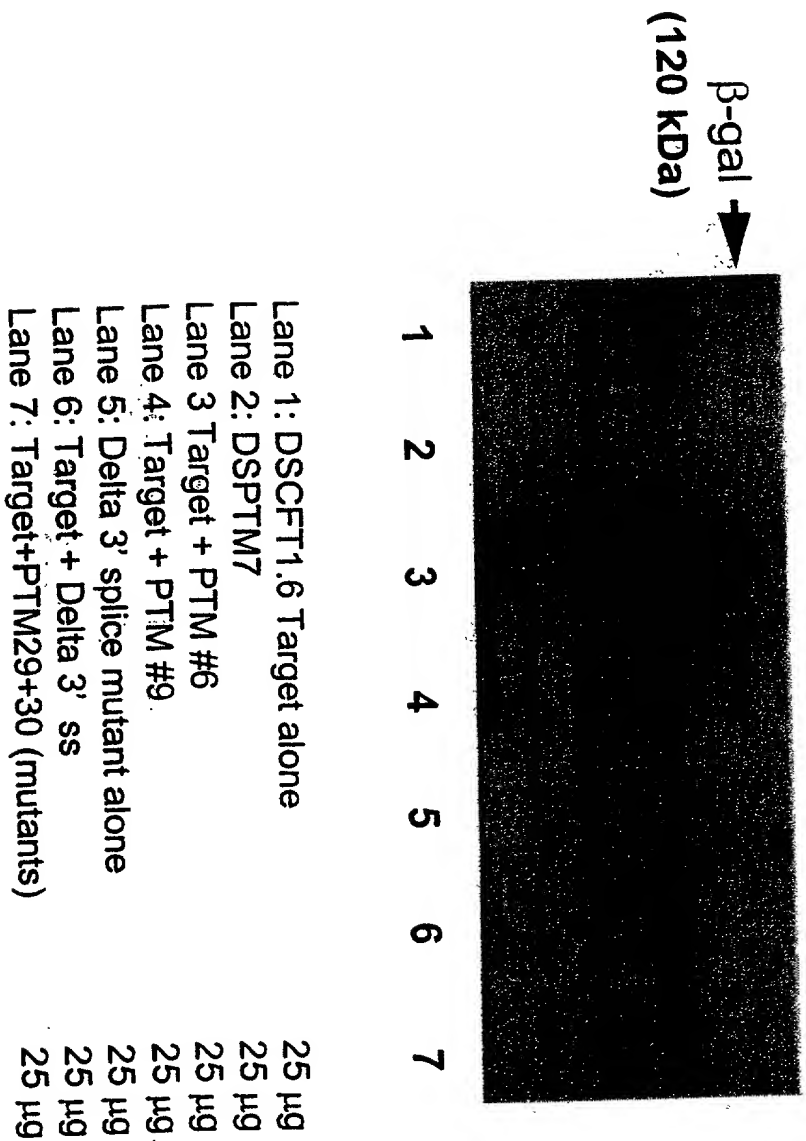


Figure 24

RESTORATION OF β -GAL FUNCTION BY DOUBLE TRANS-SPICING

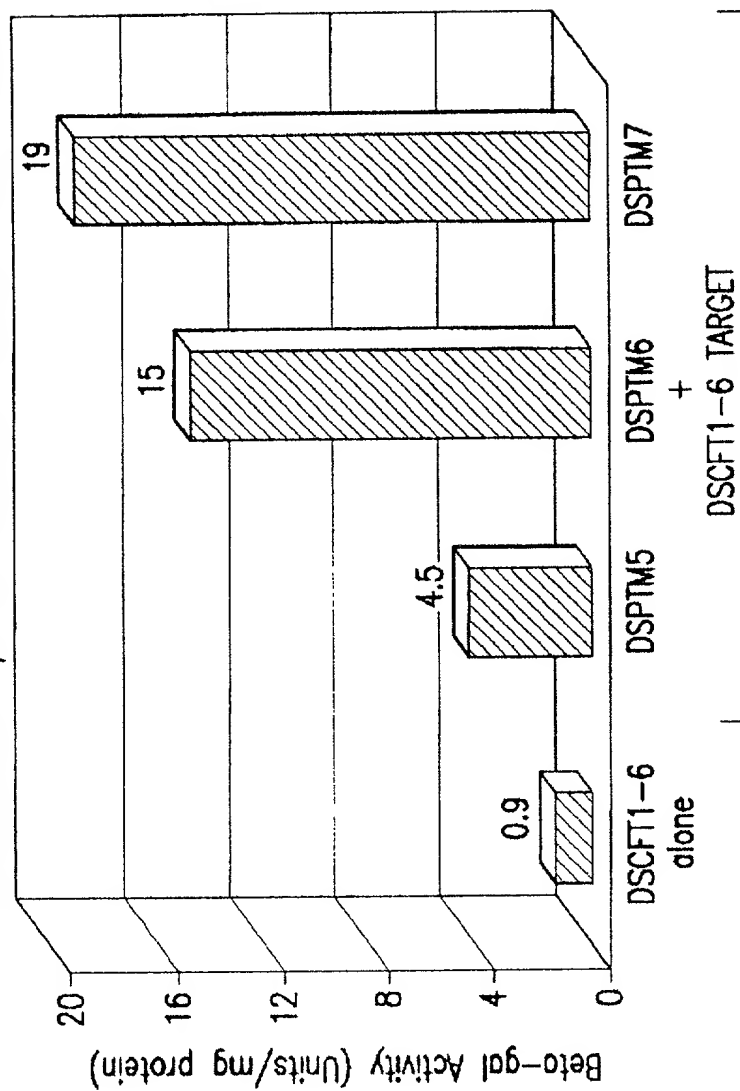


FIG.25

RESTORATION OF β -GAL ACTIVITY IS DUE TO DOUBLE RNA TRANS-SPICING EVENTS

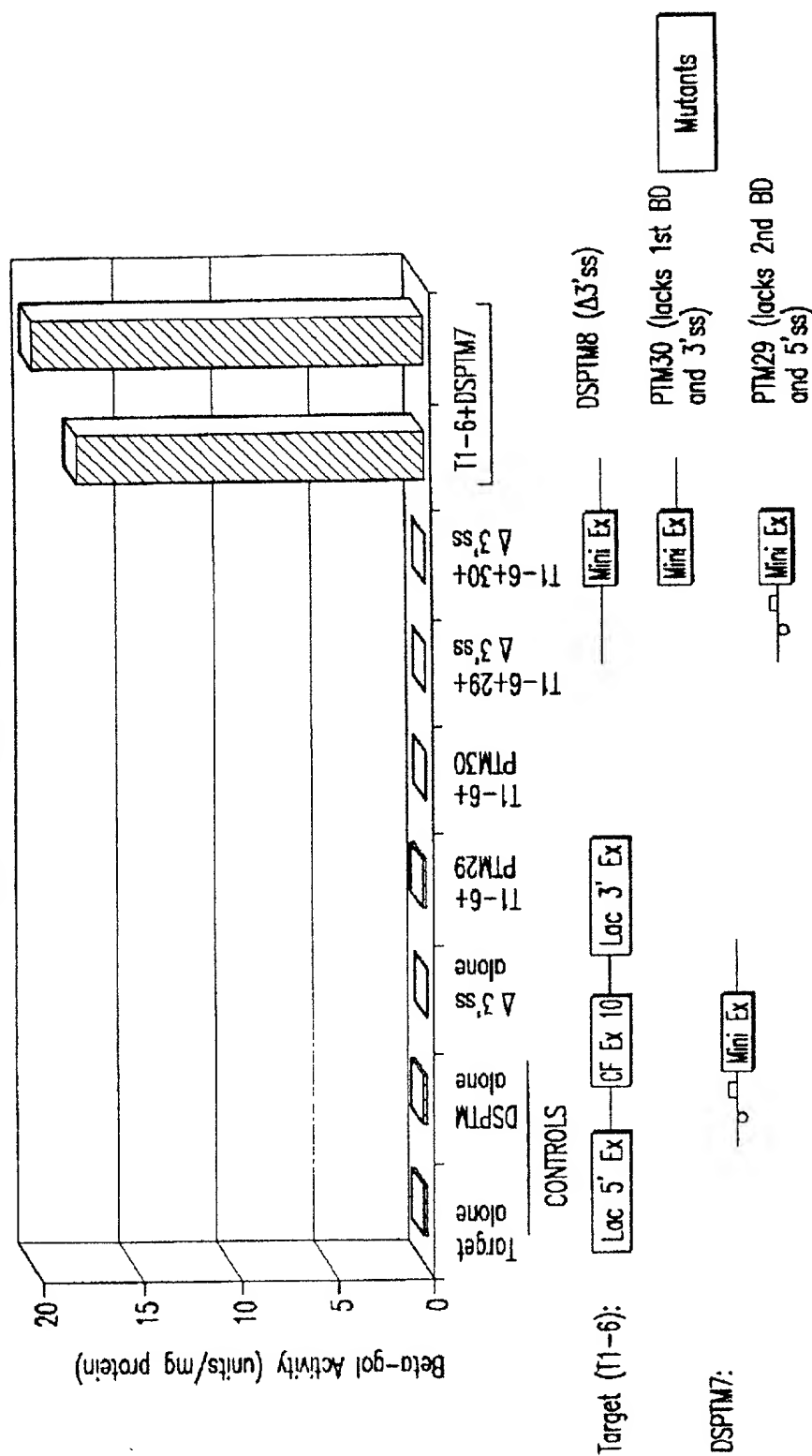


FIG. 26

DOUBLE TRANS-SPLICING: TITRATION OF TARGET & PTM

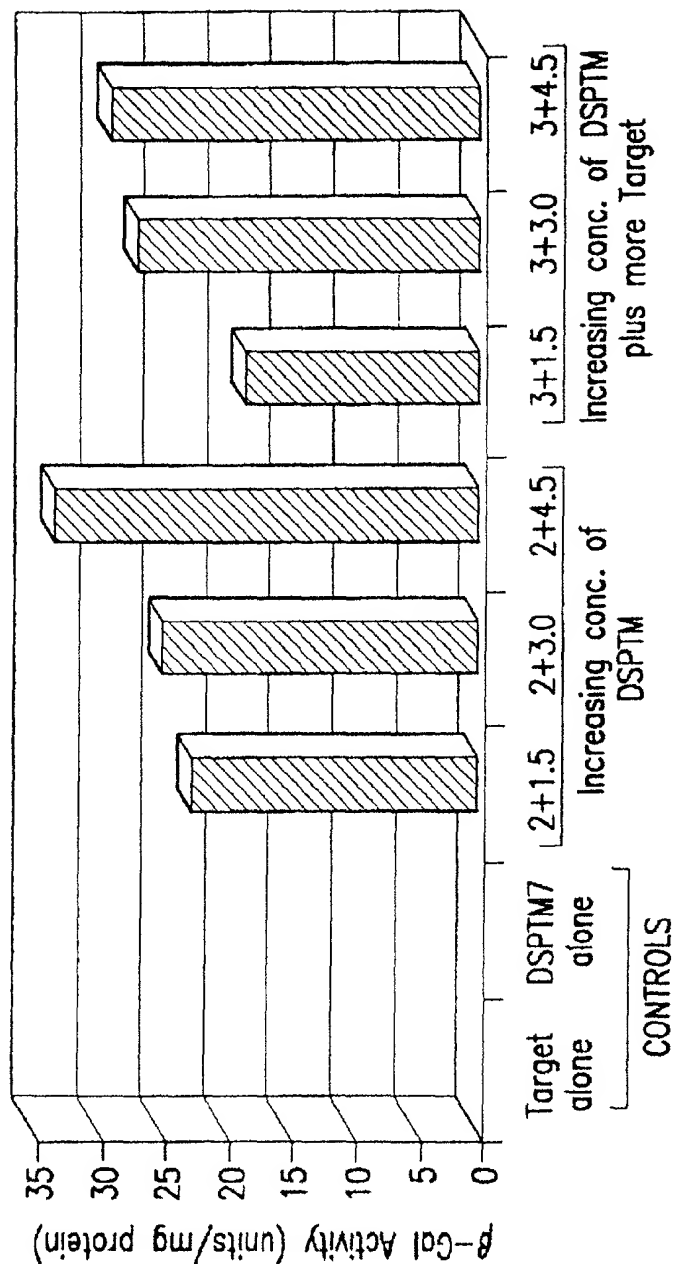


FIG.27

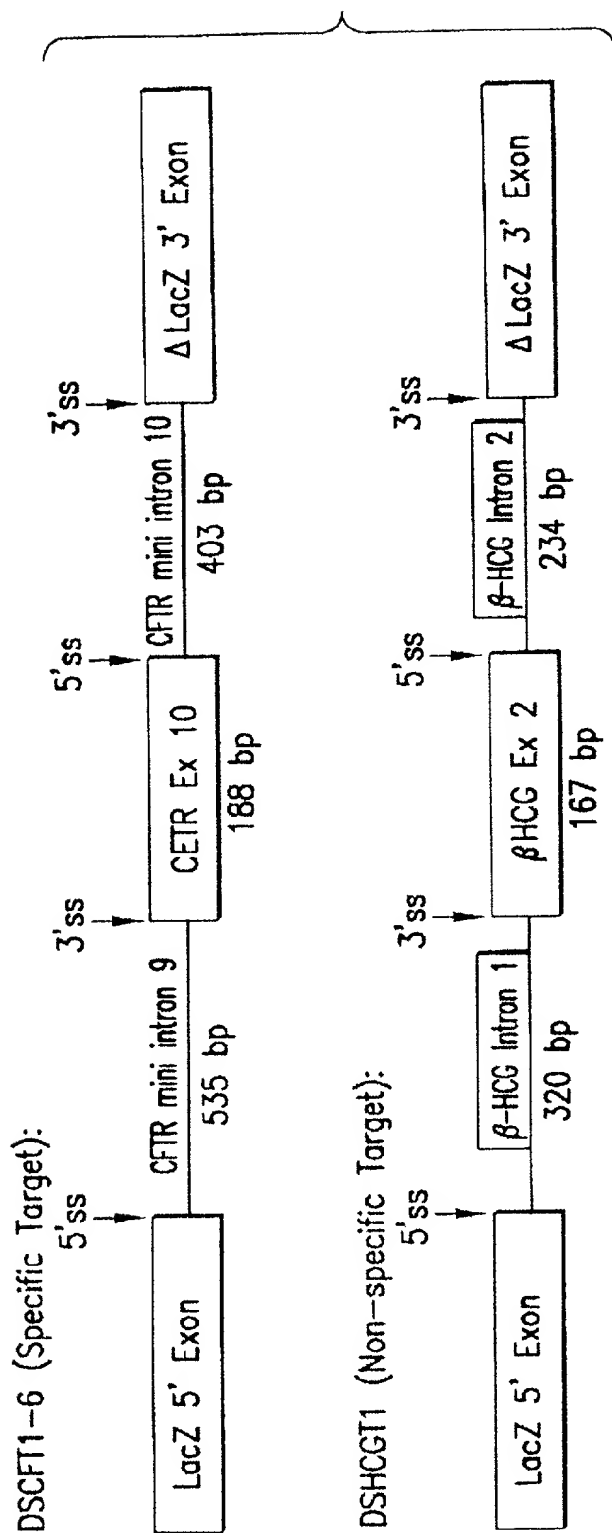


FIG.28

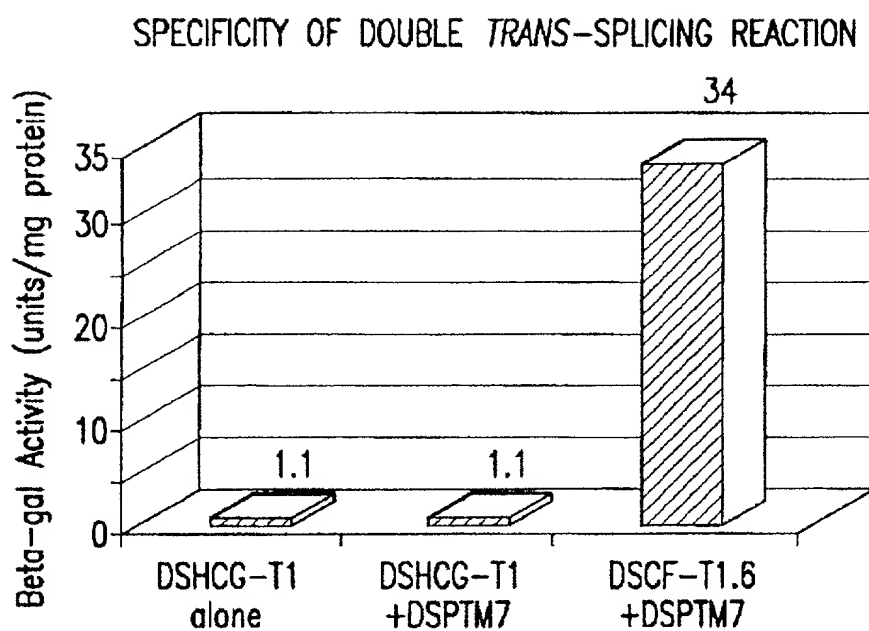


FIG.29

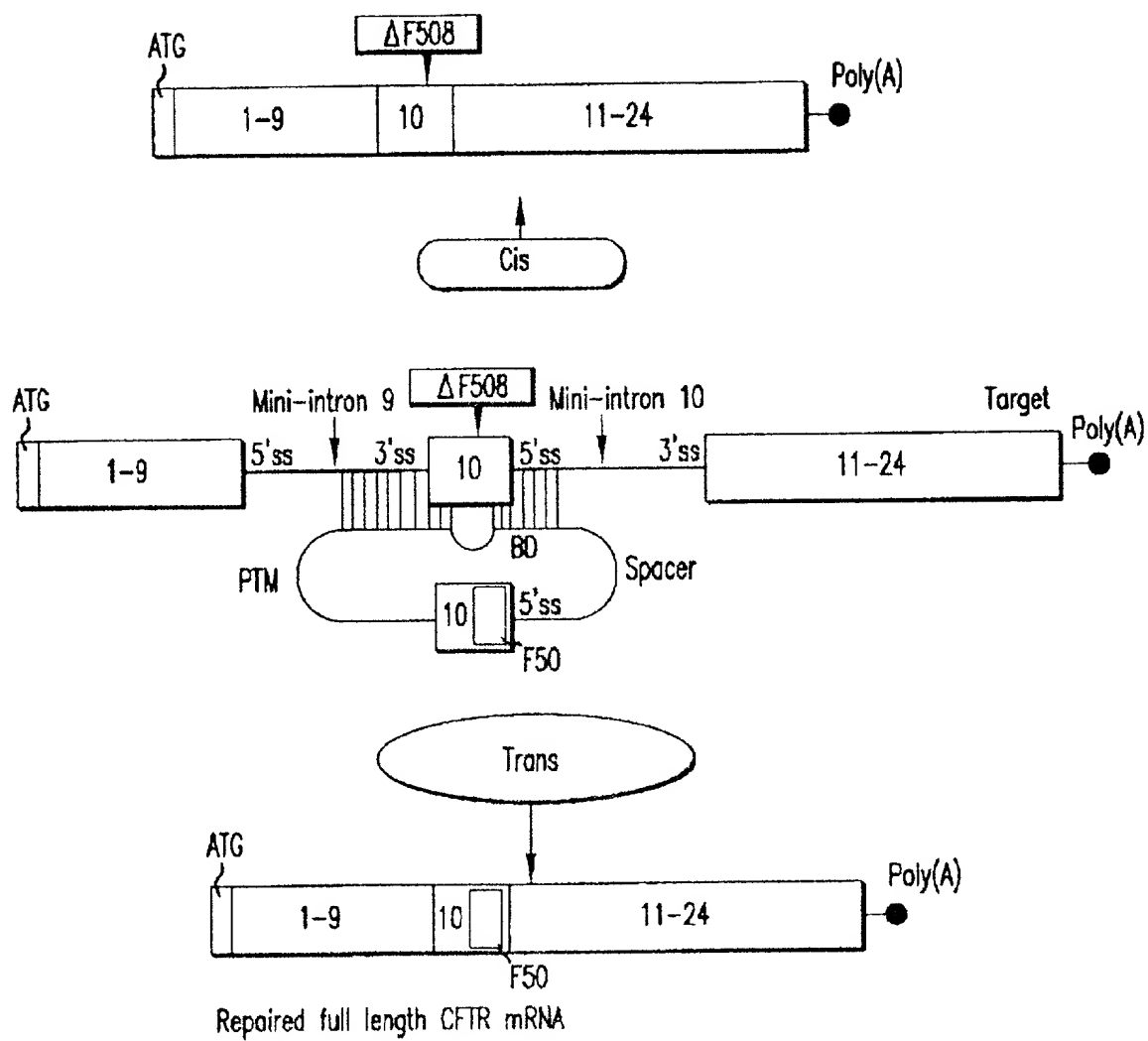
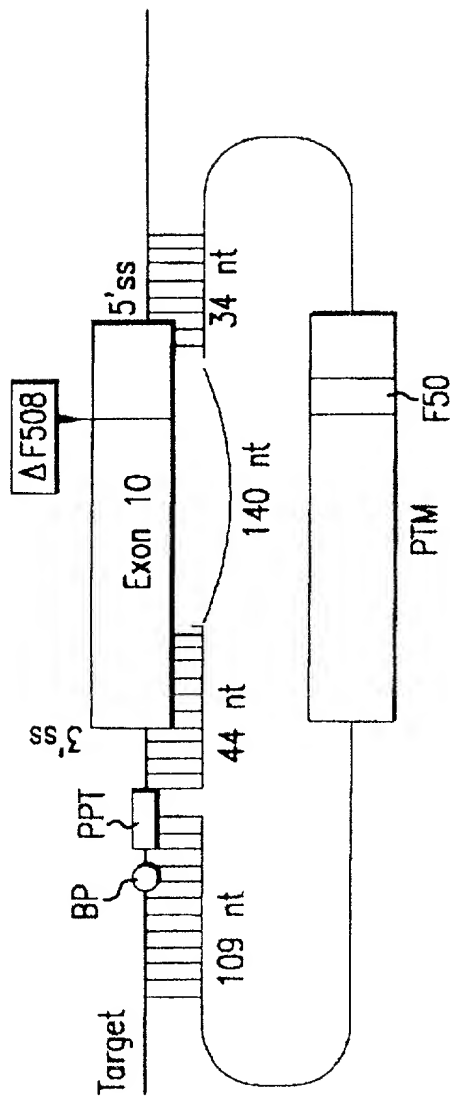


FIG.30

PTM with a long binding domain masking
two splice sites and part of exon 10
in a mini-gene target



ACGAGCTTGCATCATGATGGCGAGTTAGACCAAGTGAAGGCAAGATCAAAATTCGG
GCCCCATCAGCTTTTCAGCCAAATTCAGTTCGATCATGCCCGTACCATCAAGCAGAAATAT
CTTCGGCGTCAGTTCAGTACGACGATACCGCTATCCCTCGGTCATTAAGCCCTGTCAGTTCGAGGAG

MCU in exon 10 of PTM

88 OF 192 (46%) bases in PTM exon 10 are not complementary to
its binding domain (bold and underlined).

FIG.31

Sequence of a double
Trans-spliced product

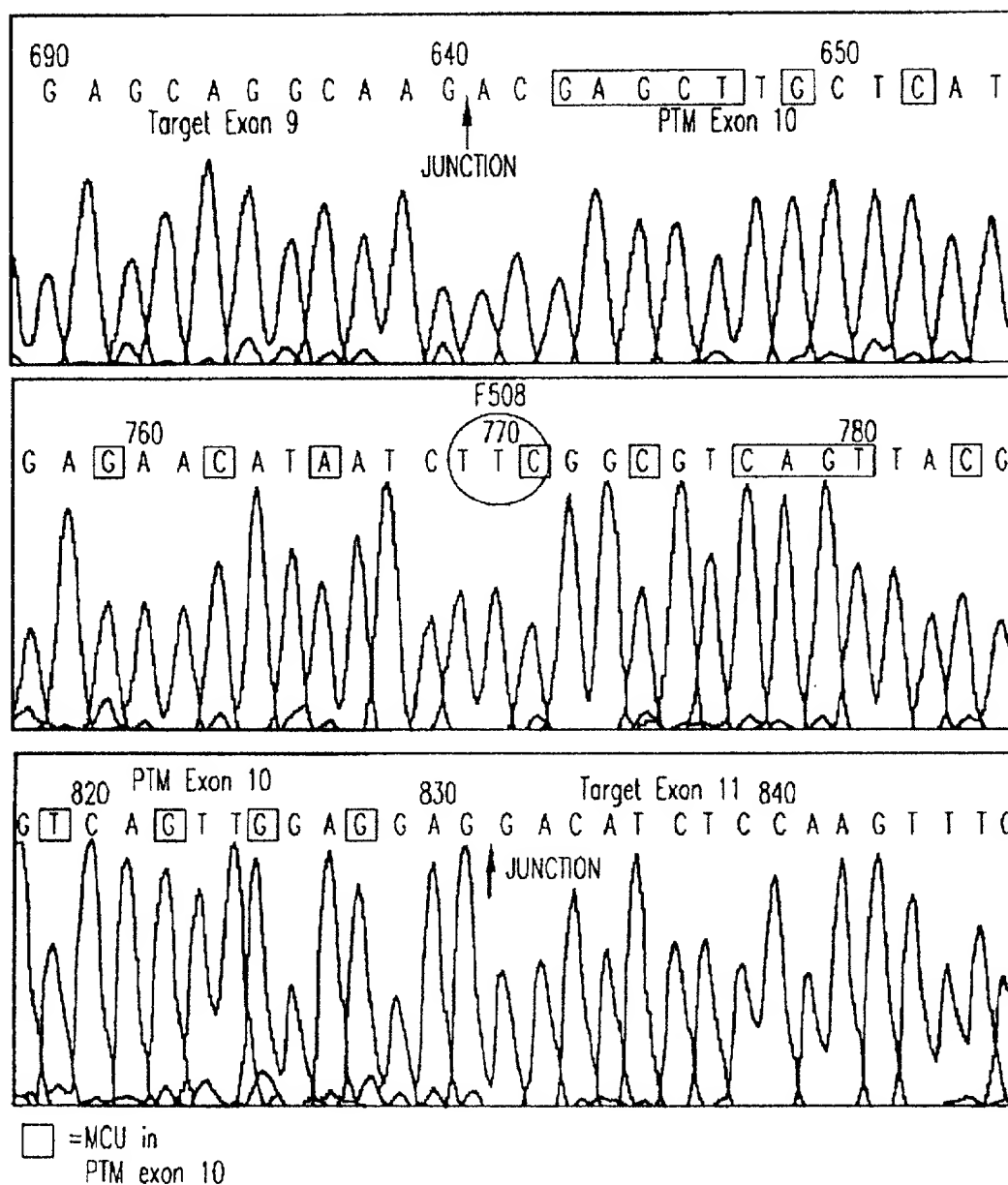


FIG.32

CF-TR Repair: 5' Exon-Replacement schematic diagram of a PTM binding to the splice site of intron 10 of a mini-gene target

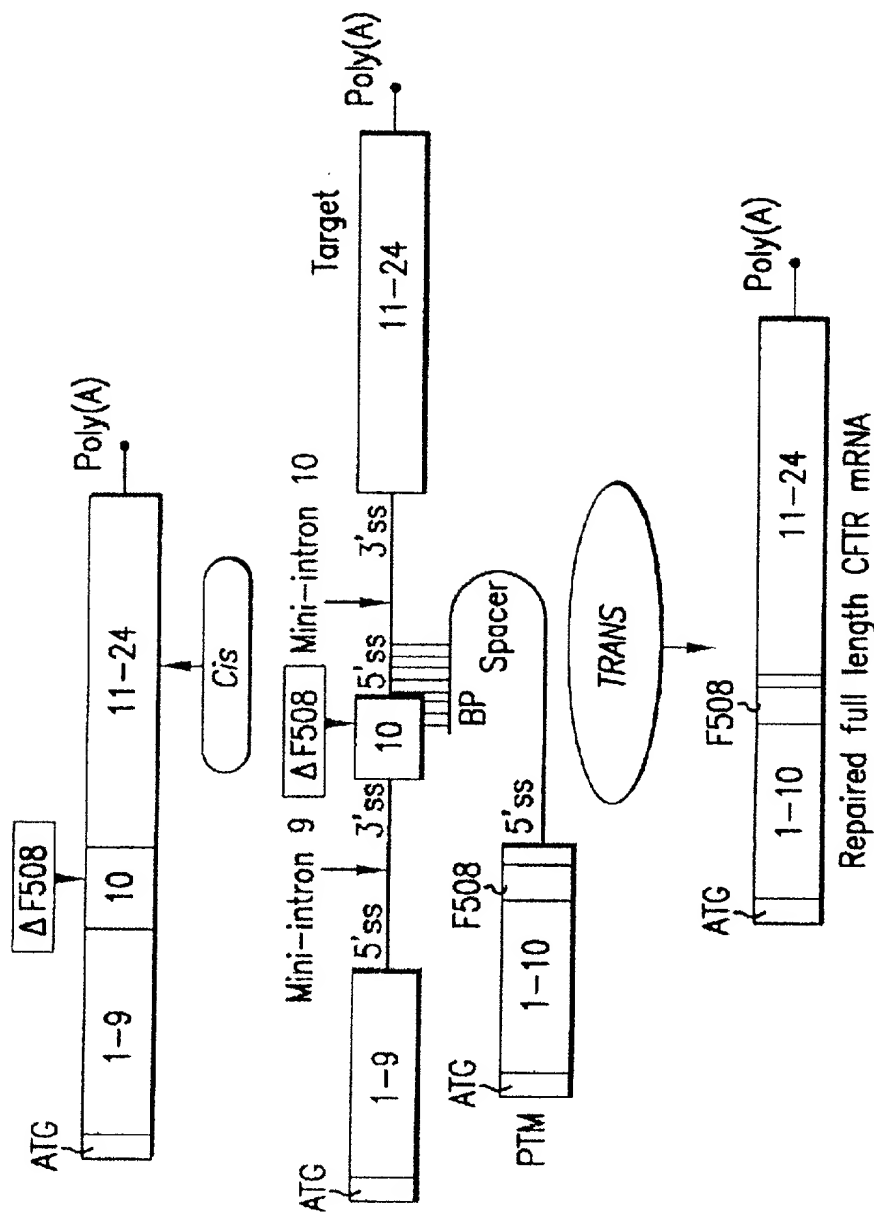


FIG.33

PTM with a short binding domain masking a single splice site in a mini-gene target.

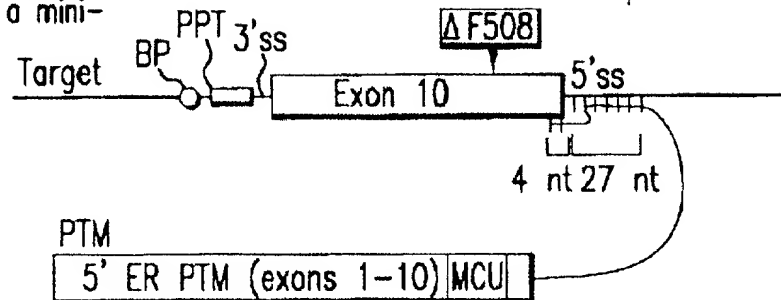


FIG.34A

PTM with a long binding domain masking two splice sites in a mini-gene target.

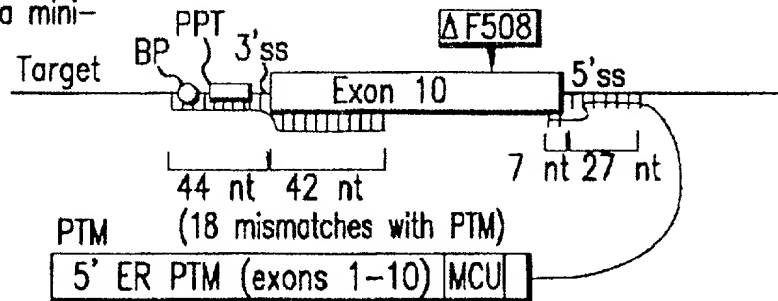


FIG.34B

PTM with a long binding domain masking two splice sites and the whole of exon 10 in a mini-gene target.

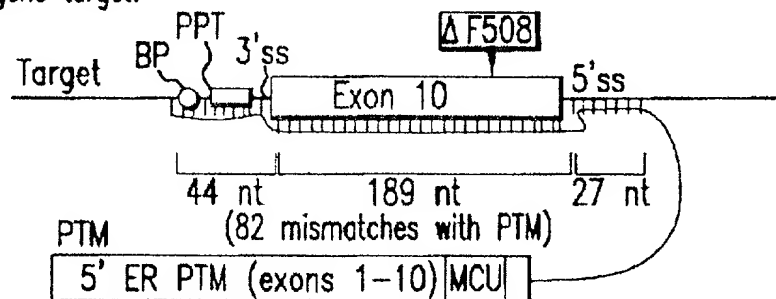
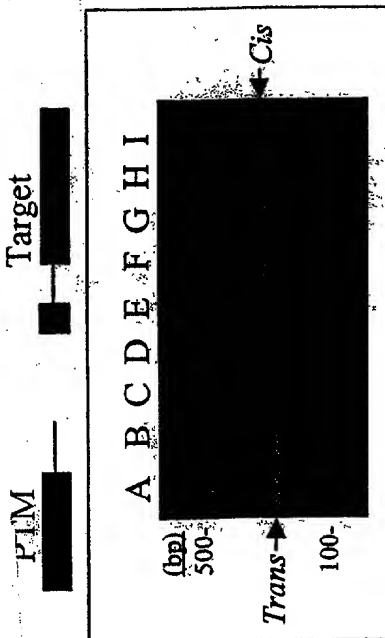
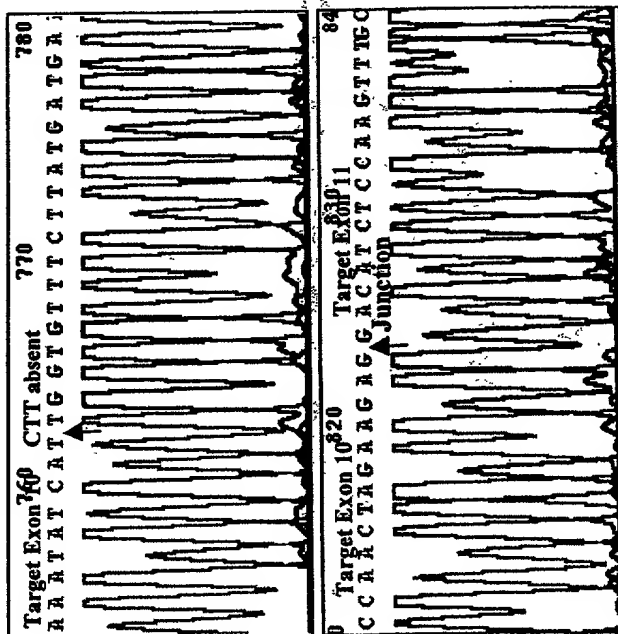


FIG.34C

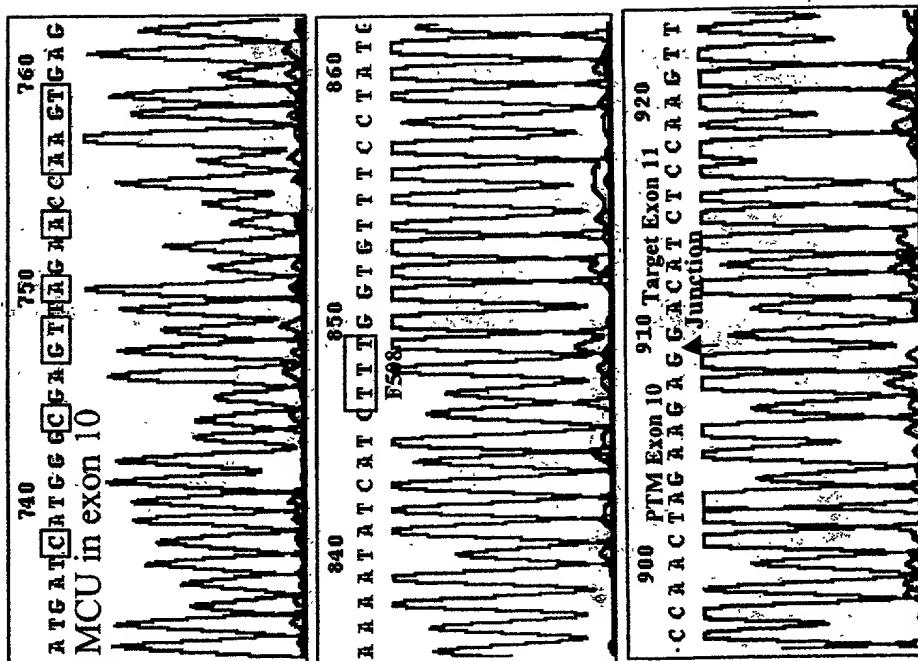
Figure 36



A.
Cis-spliced product
[Primers CF1 + CF111]



B.
Trans-spliced product
[Primers CF93 + CF111]



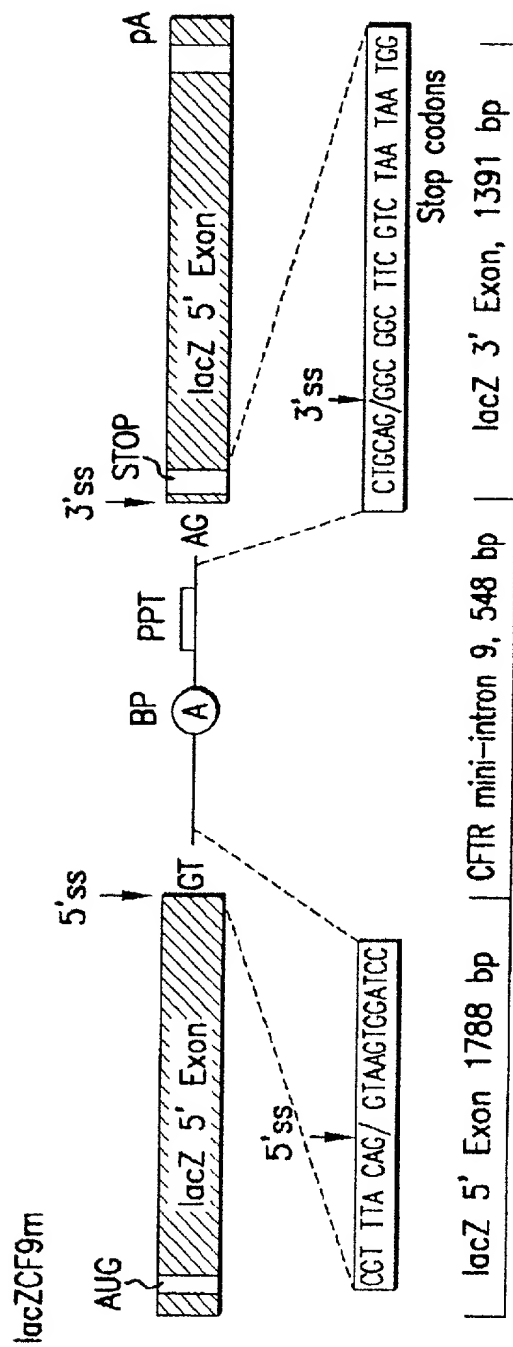


FIG.37A

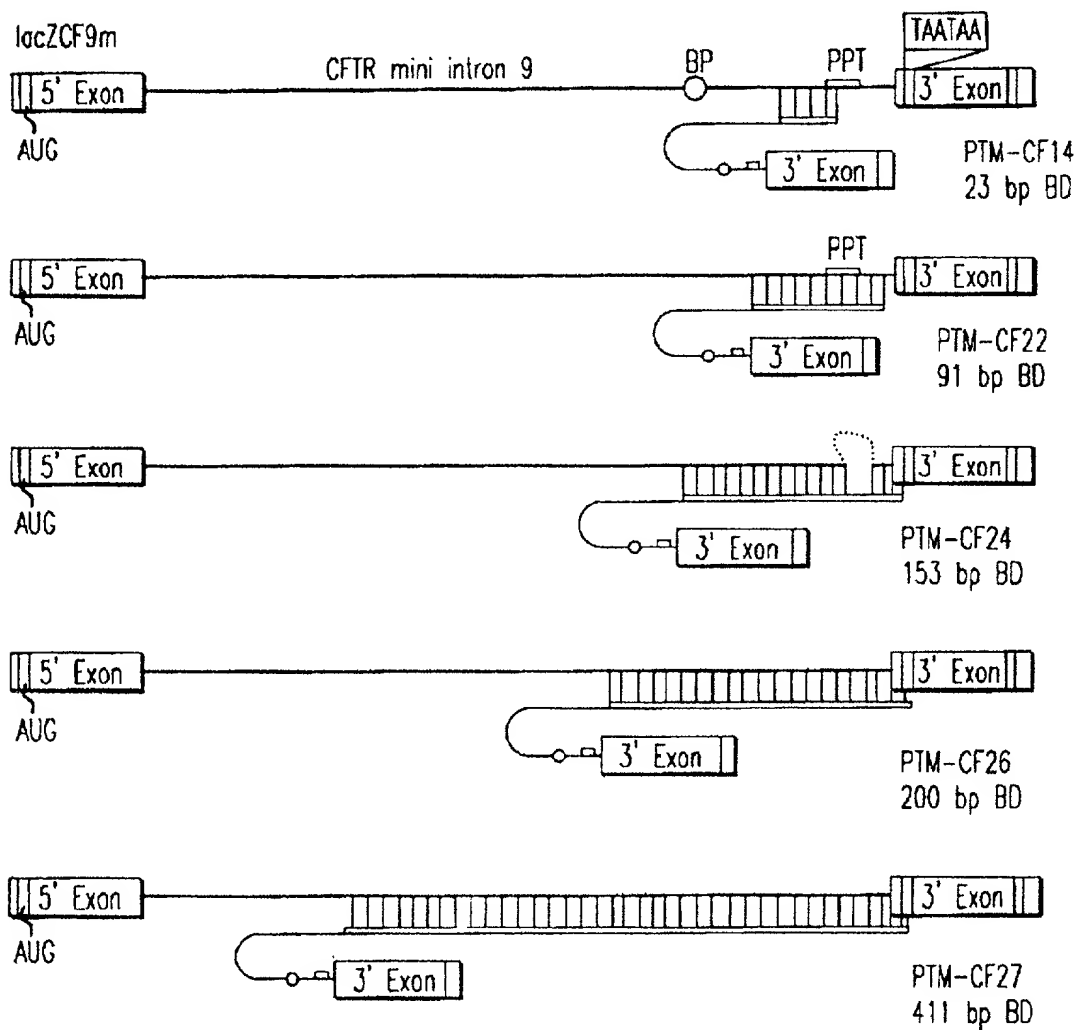
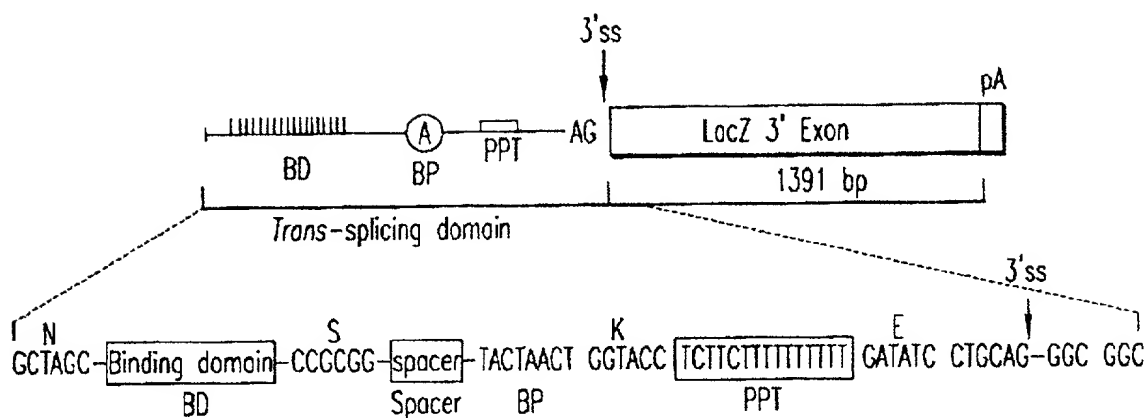


FIG.37B

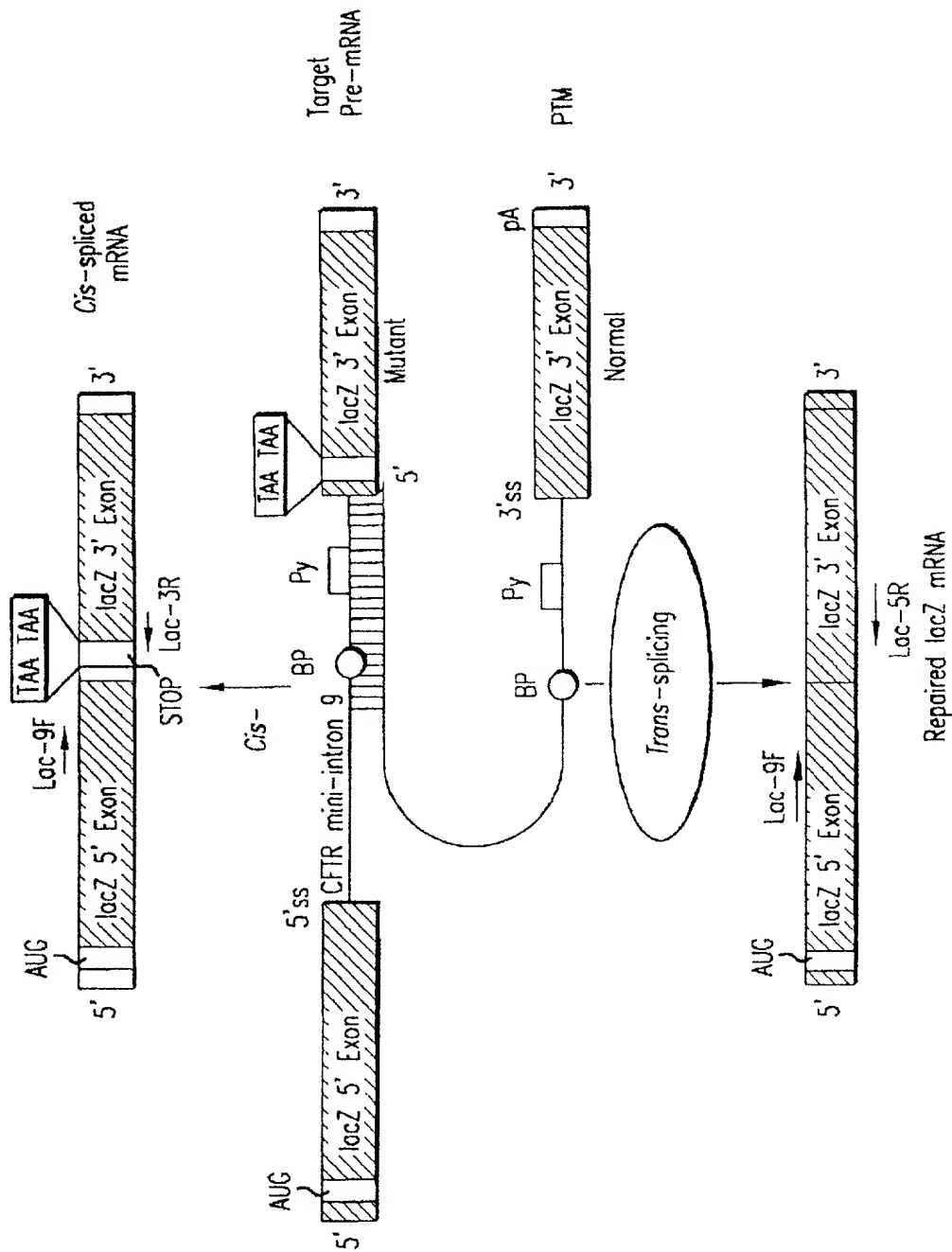


FIG.37C

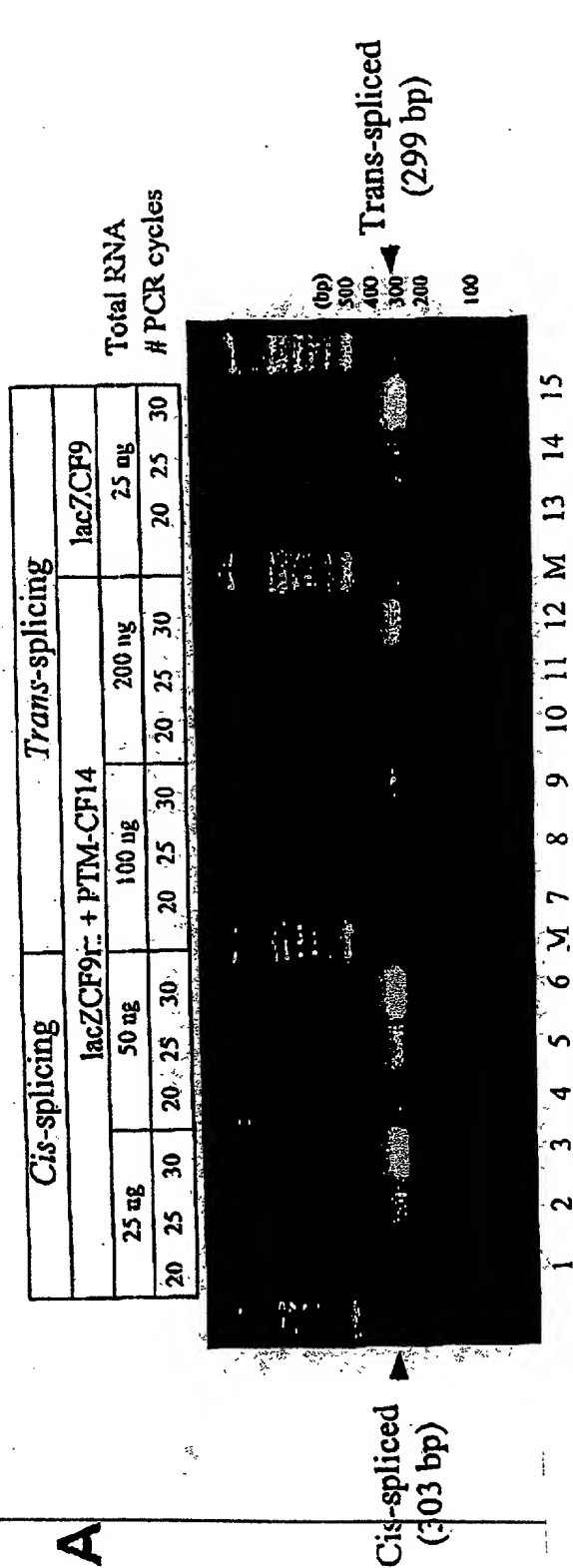
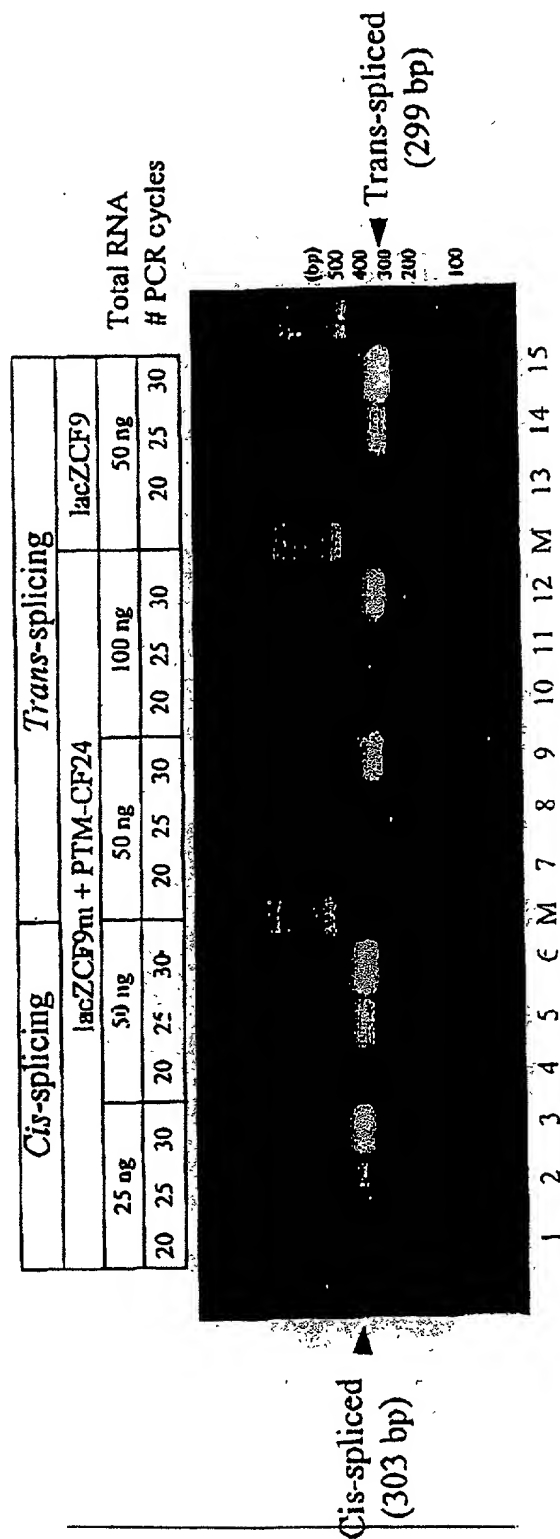


Figure 38A



B

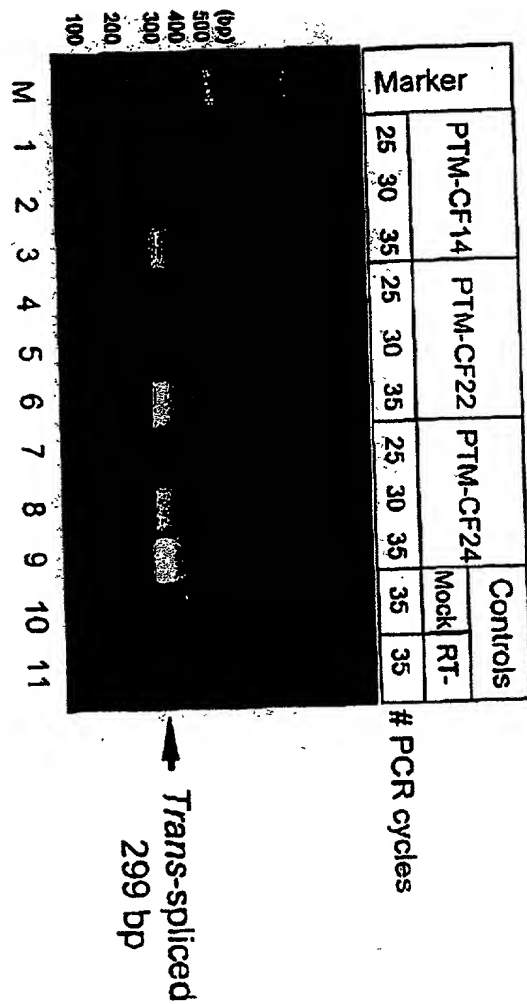


Figure 38B

A

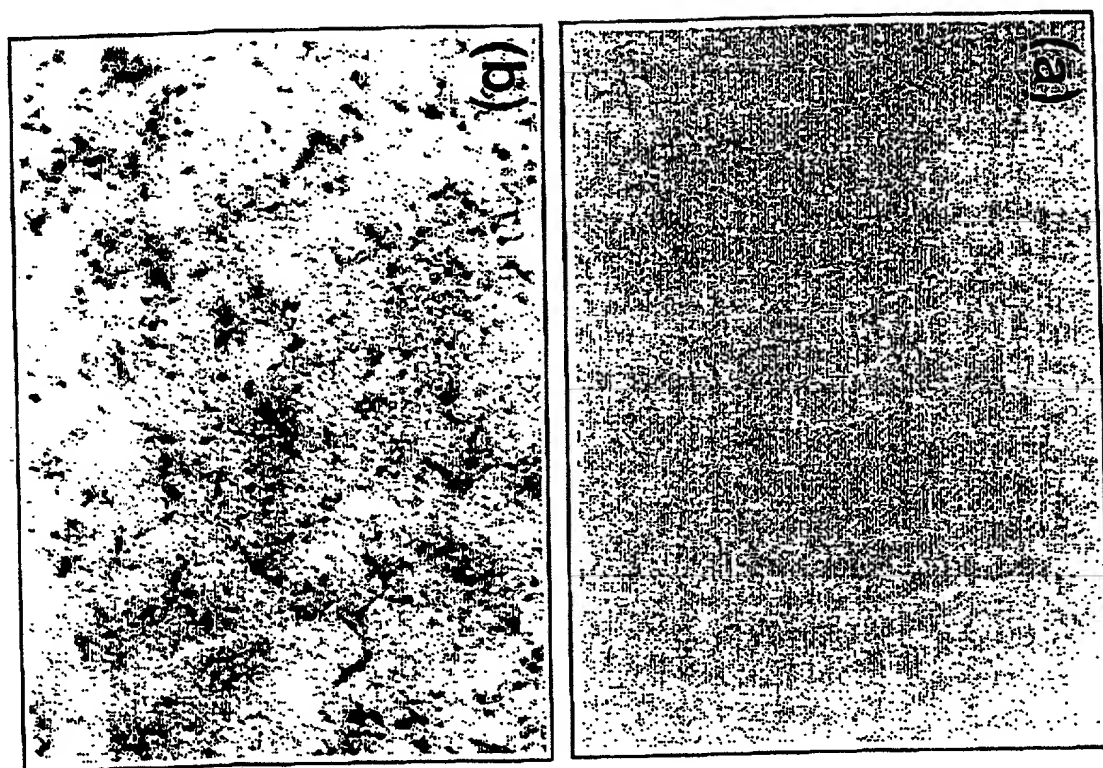


Figure 40A

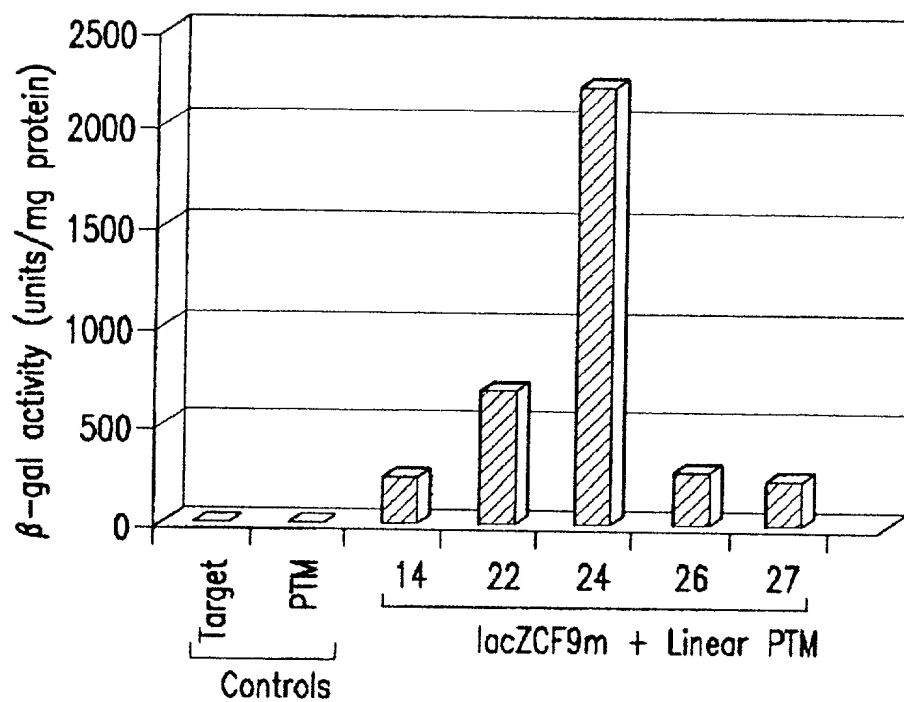


FIG.40B

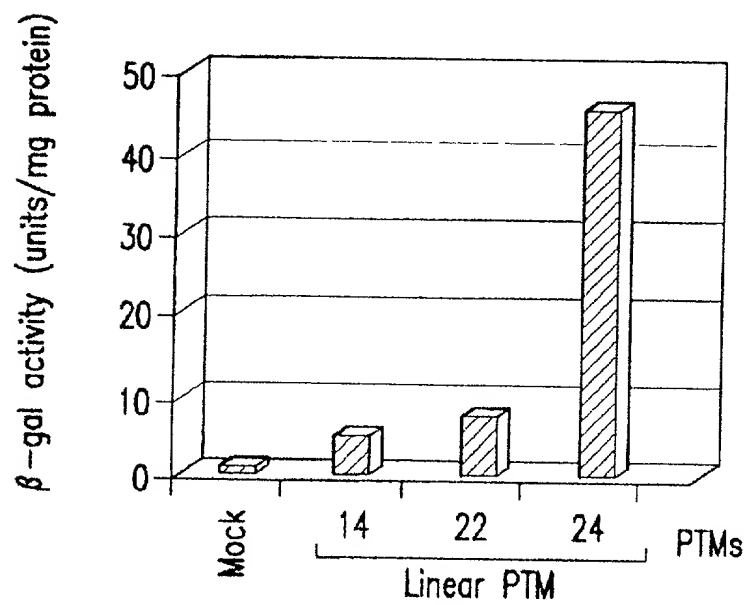


FIG.40C

20250303 14:00:00

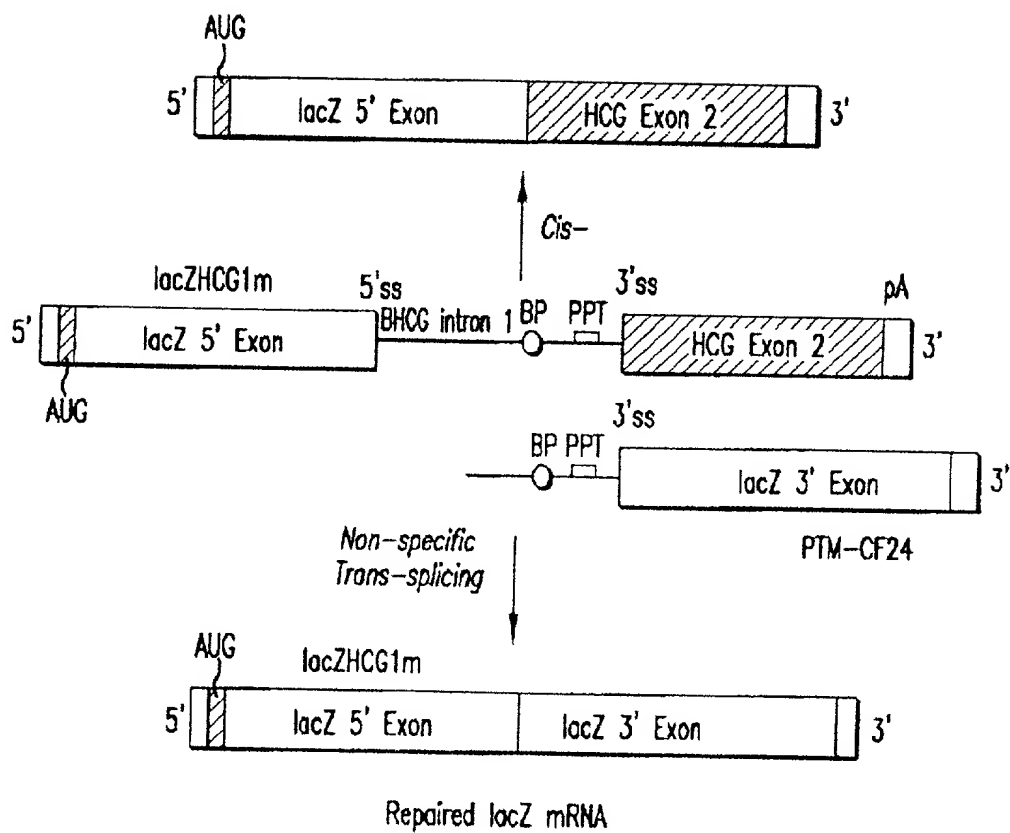


FIG.41A

四

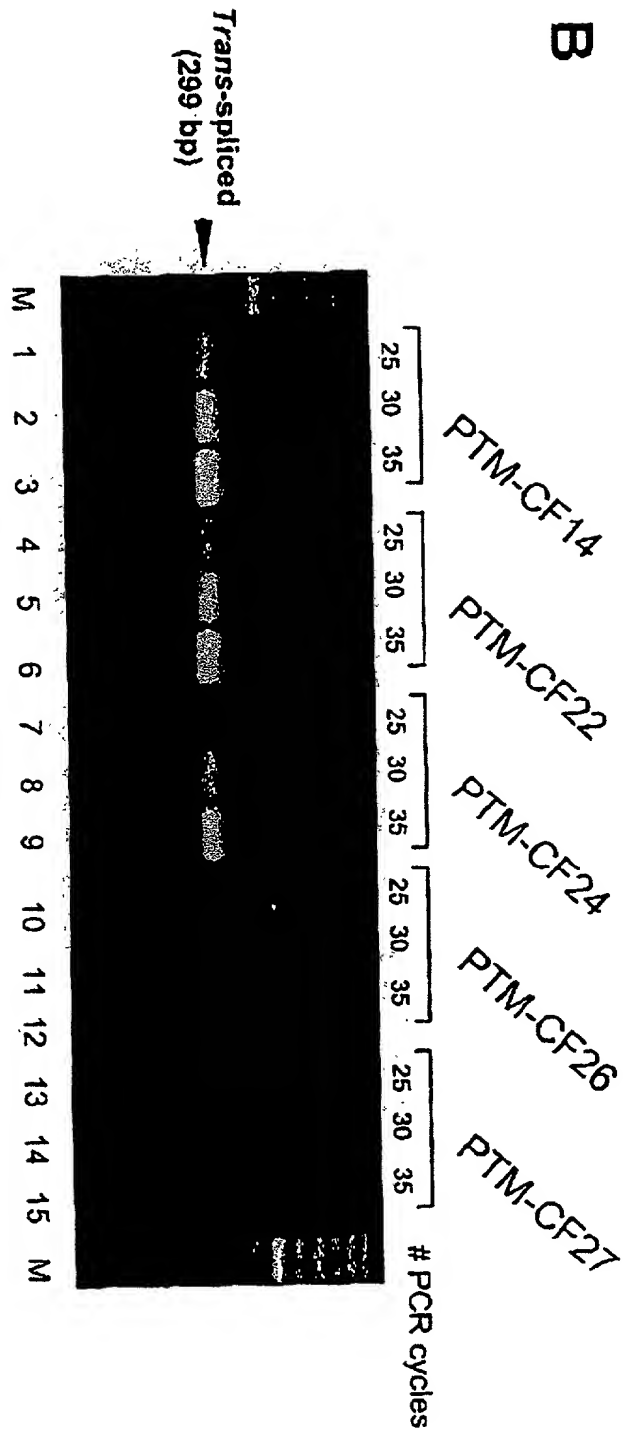


Figure 4B

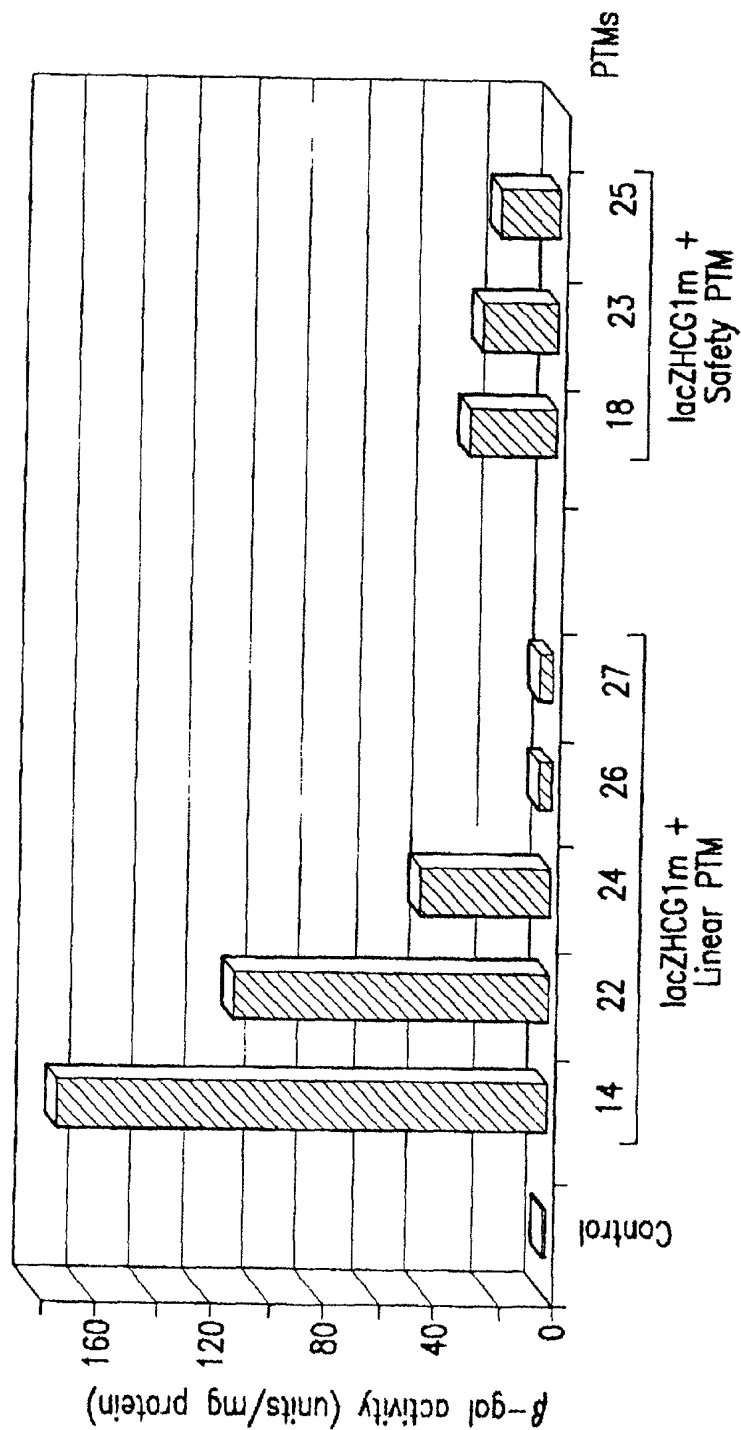


FIG.41C

Nhe I
GCTAGC-AATAATGACGAAGCGCCCTCAGCTCAGGATTCACCTGCCTCCAATTATCATCCTAAGCAGAAGTGATA
153 bp BD underlined
TTCTTATTTGTAAGATTCTATAACTCATTGTGATTCAAAATATTTAAAATACTTCCIGTTTCACCTACTCTGCTATGC

FIG. 43A

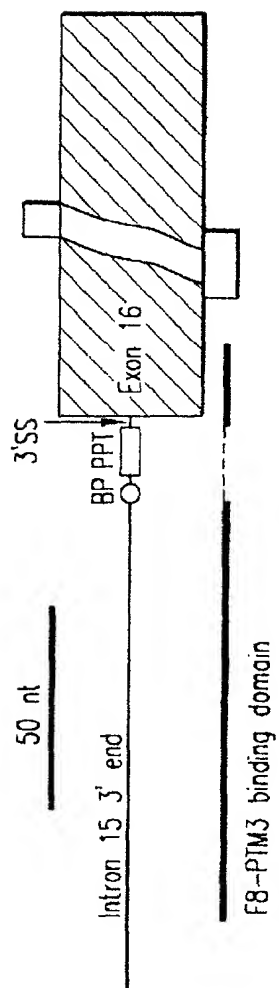


FIG.44B

[illegible]

Sequence not included in construct
 CCGCGCC TCGCG CGCGCGCGCGCGCTCTACTGACGGGTTACTCCACAGGTGAG
 CGGCGCGGACGGCCCTTCTCTCGGGCTGTAATTAGGCGCTGGTTTATCAGCGCT
 TGTCTCTTTCTGTGGCTGGTGAAAGCCCTGACGGCGCTCCGGAGGCAATTGGTA

Extent of promoter in above construct

Extent of promoter in original construct

CMV enhancer

CBA promoter

Exon 1

Intron 1 (partial)

525 277 94 117

F13+F2=235+106=341 bp
F13+F4=235+315=550 bp

Chicken Beta Actin Promoter (including exon 1 and part of intron 1)

FIG. 44C

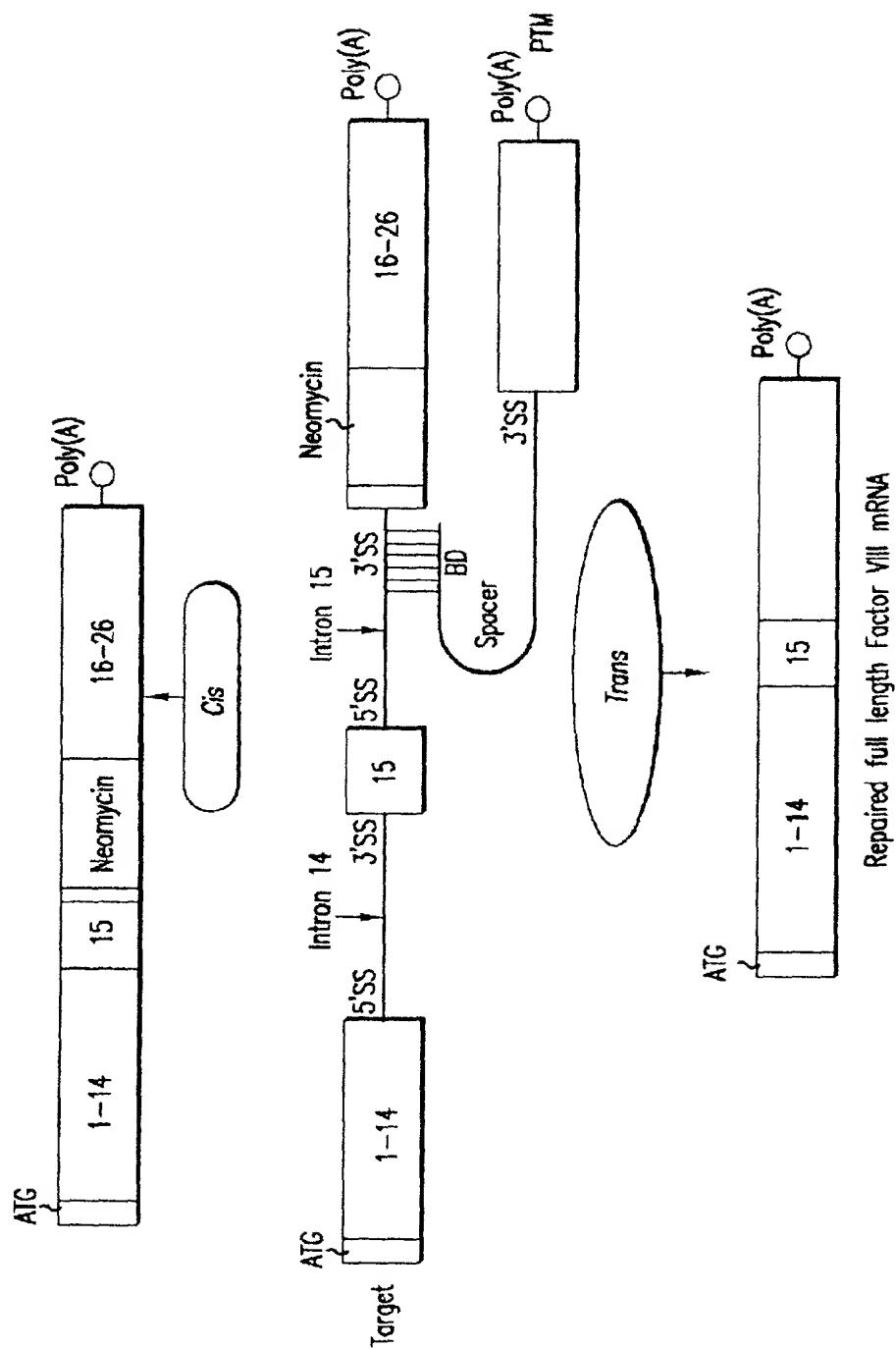
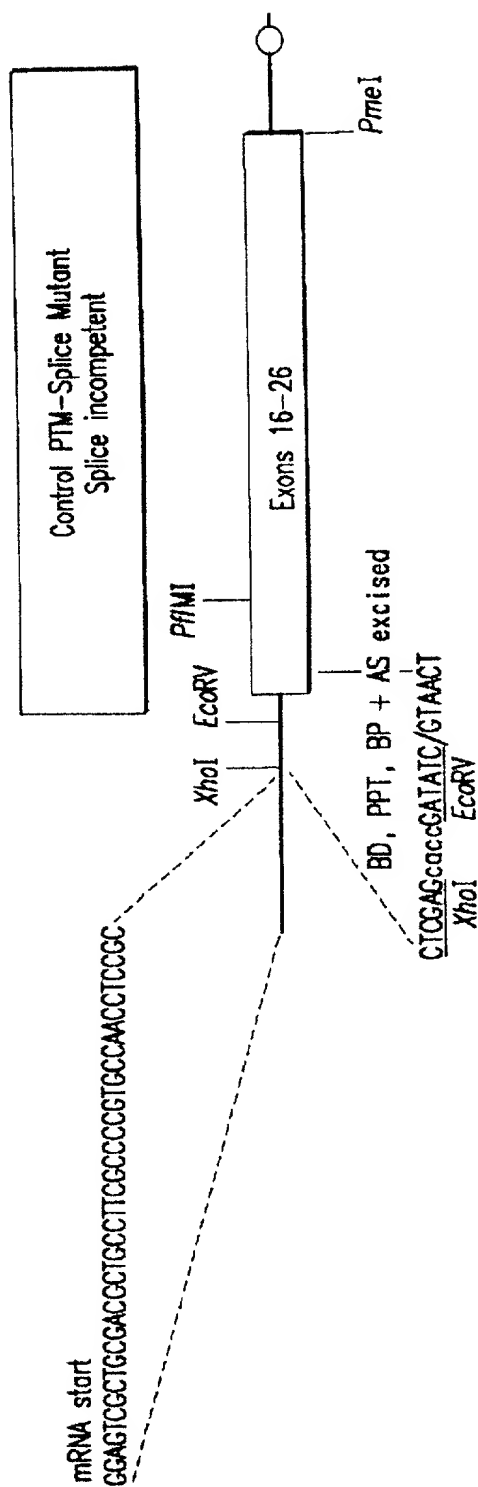


FIG.44D



Method:
Excise TSD and part of exon 16 with
XhoI and PflMI and ligate in a PCR product that:
1) eliminates the TSD and splice acceptor site
2) inserts EcoRV adjacent to exon 16
3) restores the coding for exon 16

FIG.45

Repair of Factor VIII
Preliminary results from one experiment

FVIII activity in Exon 16 FVII-KO mice
after IV PTM-FVII intraportal infusion
(100 μ gDNA)(n=3)

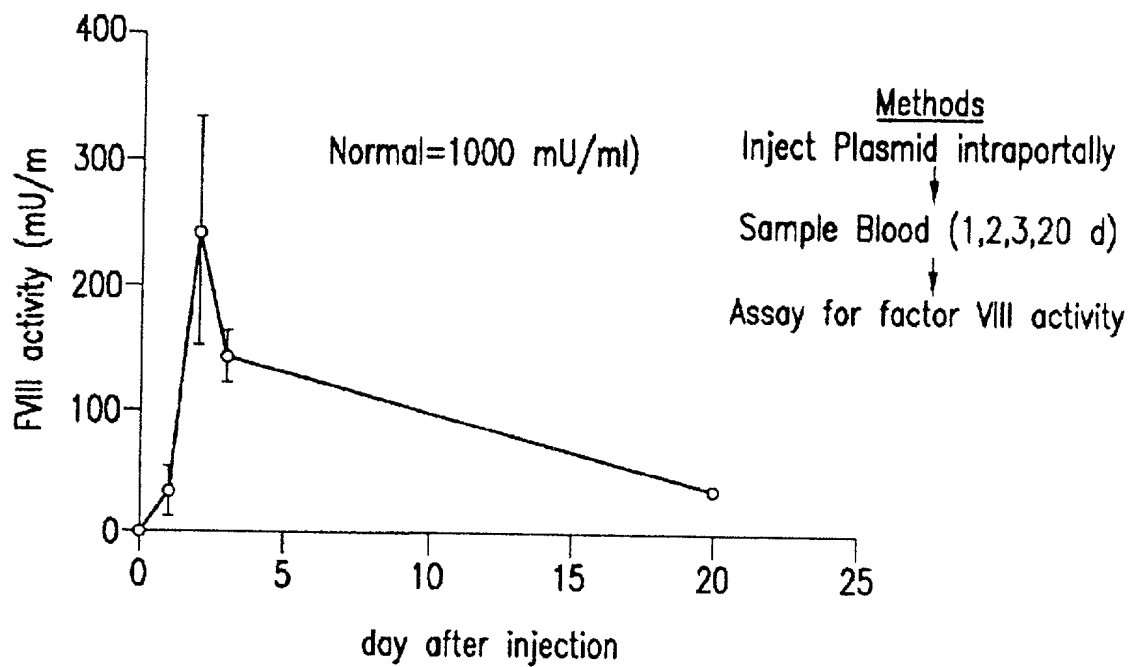


FIG.46

Detailed structure of a mouse factor VIII PTM containing normal sequences for exons 16-26 and a C-terminal FLAG tag. BGH=bovine growth hormone 3' UTR; Binding domain= 125 bp.

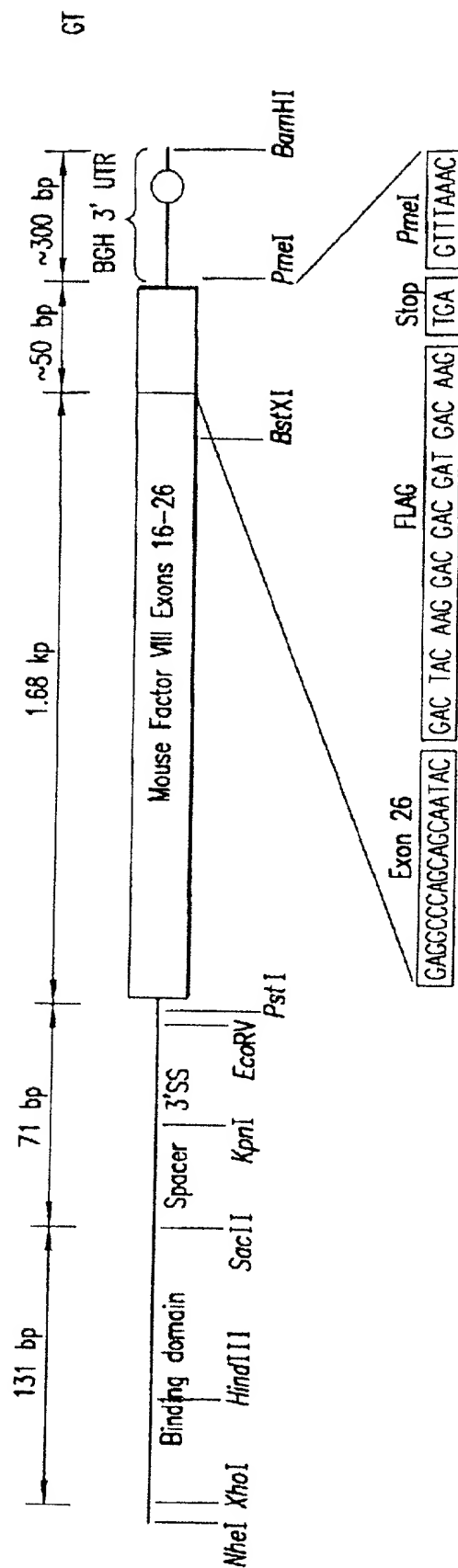
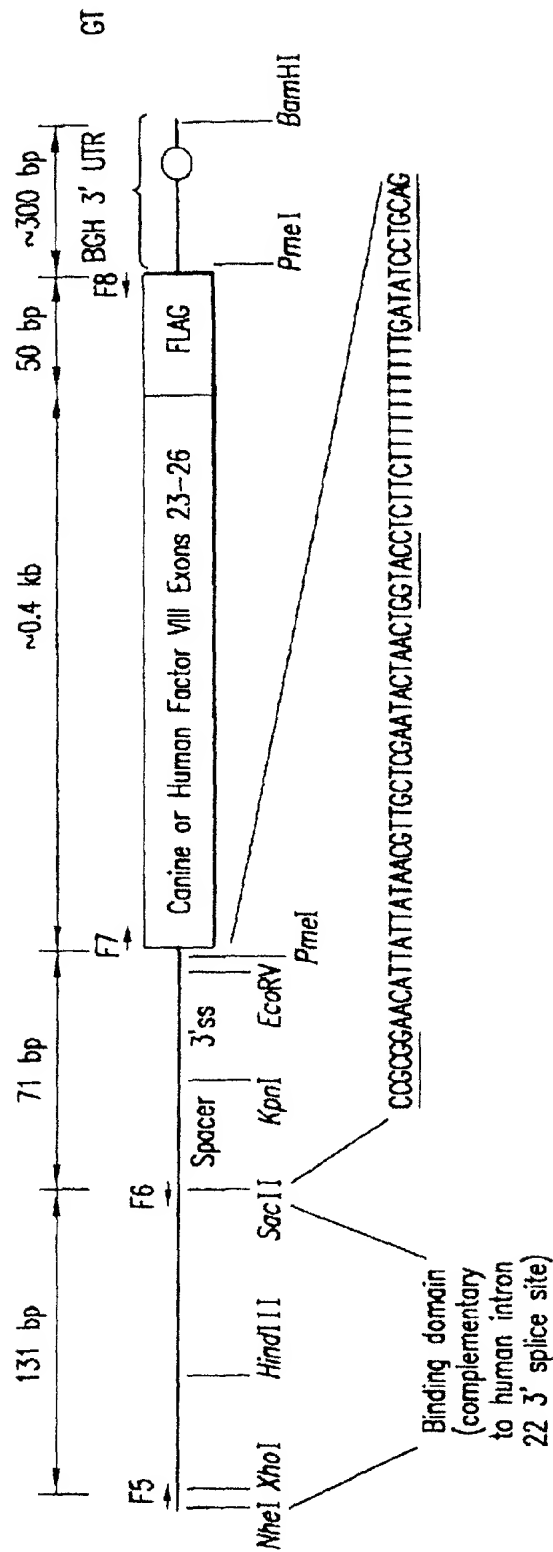


FIG.47A



FLAG=C-terminal tag to be used to detect repaired factor VIII protein.

FIG.47B